PROCEEDINGS OF THE THIRD MEETING OF IUFRO WORKING PARTY S7.03-04

DISEASES & INSECTS IN FOREST NURSERIES

Edited by

R.L. JAMES

USDA FOREST SERVICE

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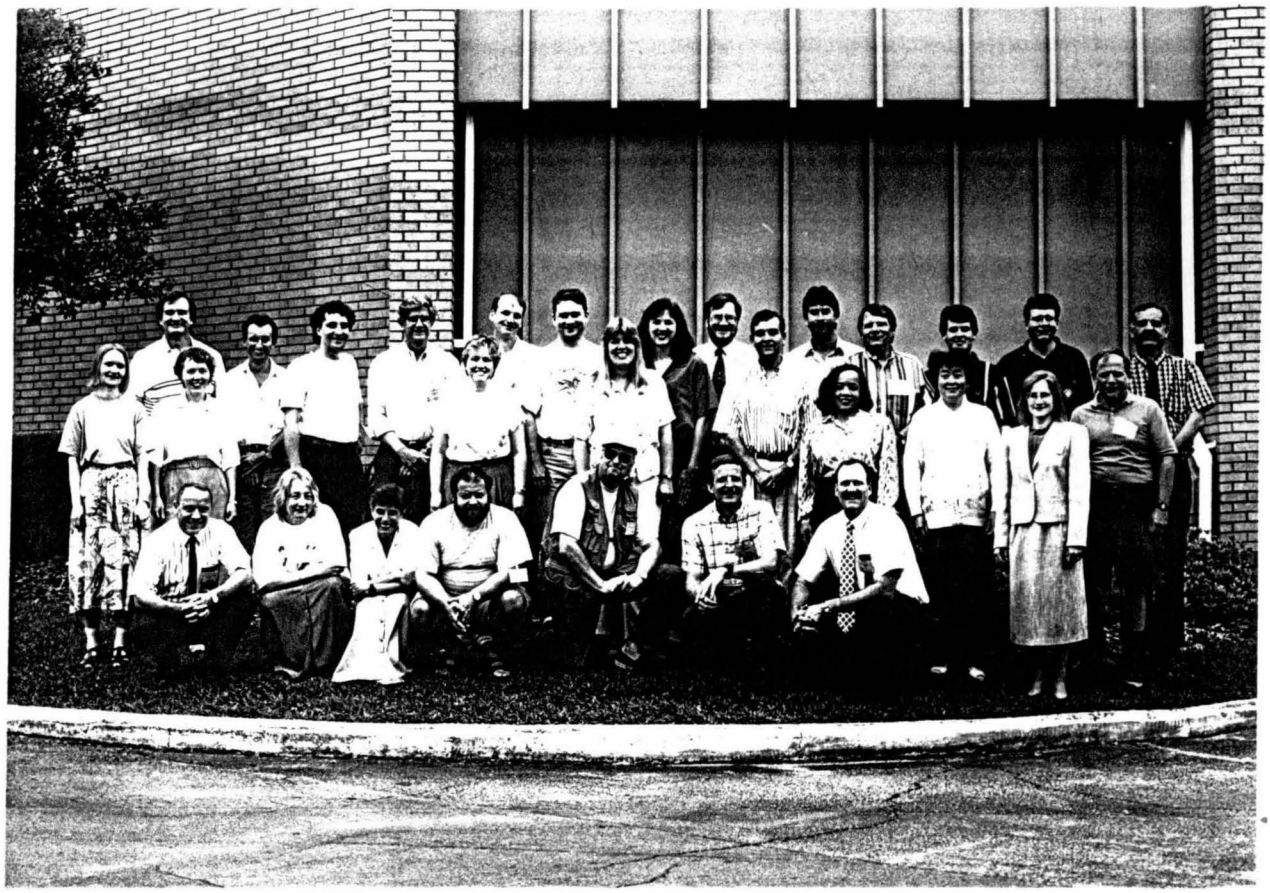

Standing (left to right): Raija-Lissa Petaisto, Robert James, Tatyana Gromovykh, Francisco Alves Ferreira, Richard Hamelin, Stanley Barras, Marie Cafourek, Steve Fraedrich, Gary Donaldson, Beth Kannwischer-Mitchell, Michelle Cram, Jeffrey Stone, Francisco Fernandez de Ana Magan, Hormilson Cruz Rios, Nominanda Fonesca, David Dwinell, Xueyan Yang, Leighton Funk, Diane Hildebrand, Bernt Arvidsson, Jack Sutherland, David South.

Kneeling (left to right): Ed Barnard, Arja Lilja, Zdenka Prochazkova, Josef Cafourek, Amaro Joao P. Barbosa, Ernie Ash, Jim Meeker.

Participants not included in photo: David Mitchell, George Blakeslee, Lisius Coehlo, Jizxi Zhou, Ying Zhou, and William Carey.

(Photo by Jeffrey Lotz, Information Specialist, Division of Plant Industry, Florida Dept. of Agriculture and Consumer Services).

MISCELLANEOUS INSECTS & DISEASES

OF FOREST NURSERIES

RECENT SUCCESS AND DISTRESS OVER SOME COMMON ARTHROPOD PESTS IN FLORIDA PINE NURSERIES[1]

J. R. Meeker[2]

ABSTRACT

A variety of historical and relatively recent arthropod pests of Florida forest nurseries continue to threaten, challenge and damage seedlings in the mid-1990's. This paper describes the nature of these insect and mite problems, and the most recently recommended and accepted tools and techniques of integrated pest management (IPM) for these pests. Experimental attempts to improve upon current control strategies are described in detail and include both successful and unsuccessful use of new insecticides and cultural practices.

INTRODUCTION

A variety of comprehensive publications already exist to provide nursery managers and others with a relatively thorough understanding of the common arthropod pests of pine nursery production in Florida (Bacon and South 1989; Cordell et al. 1989; Dixon et al. 1991; Dixon and Foltz 1984; USDA 1985). These sources also contain currently accepted practices and materials recommended to prevent, control and otherwise manage damaging impacts of these insects and mites. Despite the development, implementation and general success of the integrated pest management (IPM) strategies promoted in these and other publications, occasional and persistent pest problems continue to occur in Florida pine nurseries. Many of these problems, whether perceived or actually capable of causing significant monetary losses, occur as a result of deviating from IPM practices, inattentiveness, human error, equipment failure, lack of resources, or abnormal and undesirable weather. Unfortunately, some pest problems develop due to the inherent inadequacies of existing IPM tools and techniques to predict, evaluate and/or control arthropod populations.

Current IPM strategies for the most common pests of pine nurseries in the South generally lack important and reliable components necessary to achieve their potential effectiveness such as economic treatment thresholds, accurate predictive capabilities, and definitive risk/hazard rating systems. Another present-day weakness of some IPM practices is that even appropriate treatments with the "latest and best" available technique and product sometimes fails to produce complete control or desired results and benefits. In addition, the costs, risks, and negative ramifications associated with using methods and materials proven to effectively control pests, may be prohibitive or undesirable. Given these deficiencies, nursery managers continue to covet cheaper, safer, more effective and labor-saving means of coping with the established group of potential pests of pine nurseries in Florida.

The arthropods generating recent concerns to pine nursery production in Florida include: plant bugs (Homoptera: Miridae, *Lygus lineolaris* (Palisot de Beauvois) and probably *Taylorilygus pallidulus* [Blanchard]), spider mites (Acari: Tetranychidae, *Oligonychus* spp., *Tetranychus urticae* [Koch], and others), white grubs (Coleoptera: Scarabaeidae, *Phylophaga* spp. and others), the lesser cornstalk borer (Lepidoptera: Pyralidae, *Elasmopalpus lignosellus* [Zeller]) and regeneration weevils (Coleoptera: Curculionidae, *Hylobius pales* [Herbst] and *Pachylobius picivorus* [Germar]). The following describes and examines some recent problems with these arthropods and attempts to control or improve existing IPM strategies.

[1] Paper presented at the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). Orlando-Gainesville FL., USA. May 19-24, 1996.

[2] Florida Department of Agriculture and Consumer Services, Division of Forestry, Gainesville, FL, USA.

PLANT BUGS

Various species of plant bugs (Hemiptera: Miridae) have relatively recently been recognized as important pests of conifer nurseries throughout the world (South 1991). Plant injury occurs during piercing and sucking of sap from the growing tips of young tender seedlings by the adults and nymphs of these small bugs. This feeding commonly causes multiple-leaders (often referred to as "bushy-top") and other disfigurements or damage, which, when severe, hinders seedling survival (Dixon 1989). The tarnished plant bug, *Lygus lineolaris*, and more recently another species, *Taylorilygus pallidulus*, have been shown to cause considerable damage of this sort in southern pine nurseries (South 1986; South and Zwolinski 1993). Prior to, or in the absence of control efforts, bushy-top and associated symptoms of plant bug damage had become increasingly important since first noted in 1982 (Dixon 1989; South 1991; South et al., 1993). Recent elevation of these cosmopolitan and polyphagous insects to prominent pest status in conifer nurseries is presumably due to the abandonment of xylene and mineral spirit treatments for weed control (South 1991; South and Zwolinski 1996).

Before control efforts were developed and utilized, there were reports of nurseries in the south experiencing 17% (Bryan 1989), 30% (South and Zwolinski 1993) and even 50% (Dixon 1989) of their seedlings being damaged by plant bugs, and associated culling of 10% of their crop (South and Zwolinski 1996). In Florida, damage is most severe and prevalent on Choctawatchee sand pine (*Pinus clausa* (Chapm.) Vasey var. immuginata Ward) followed by loblolly pine (*P. taeda* L.), and slash pine (*P. elliottii* Engelm.). Significant damage from plant bug feeding predominantly occurs shortly following seed germination in mid-May through June, after which seedlings become more tolerant of injury (Bryan 1989). Recent IPM tactics to reduce plant bug damage include: 1) cultural practices or insecticide applications to remove, eliminate or treat surrounding weedy vegetation, which serves as primary or alternate insect hosts (applications are most effective in winter and spring when adult overwintering and egg-laying occurs); 2) weekly or biweekly, overhead spray applications of either dimethoate, fenvalerate, or esfenvalerate from the first sign of germination through the later half of June (Bryan 1989; South 1991).

These methods and insecticides, although economically justifiable (South 1996) and providing adequate prevention of plant bug damage and associated losses, are still costly in terms of labor, equipment, materials, and time necessary to perform the 6-12 required insecticide applications. Multiple spraying of these relatively broad spectrum insecticides also increases risk of potentially undesirable consequences such as health and safety problems, human error, equipment failure or damage to seedlings, and non-target insecticidal activity.

Recent success of seed soaks with imidacloprid against a variety of sucking insect pests (including plant bugs) of various agricultural food crops (Mullins 1993), prompted field trials in Florida pine nurseries. Imidacloprid, a relatively new chloronicotinyl insecticide, has a novel mode of action with excellent systemic qualities, good residual efficacy, low vertebrate toxicity, favorable environmental fate characteristics, broad spectrum activity and crop applicability. As a seed soak, this insecticide offers particularly desirable qualities for applicators and the environment (Mullins 1993).

The Florida Division of Forestry conducted initial imidacloprid seed soak trials in 1991, but due to a variety of circumstances these were inconclusive (Dixon et al., unpublished). In a second attempt during 1992, Dixon and others (unpublished) developed and implemented the following protocol with some measurable success. Utilizing the flowable formulation of imidacloprid (Bayer Corp., Specialty Products, Kansas City, MO) approximately 0.45kg of Choctawhatchee sand pine seed were soaked for 1 hr. in diluted solutions corresponding to 0, 118, 236, and 473ml a.i. per 45kg of seed per 189L of H_2O. Seeds were then drained and placed in cold storage about 1 week until sowing of 0.02ha treatment blocks. No further insecticide treatments were applied. Final observations taken 36 days after sowing suggested that imidacloprid seed soaks were efficacious. Five samples were taken per treatment; each sample consisted of all seedlings occurring within a randomly placed 0.37m^2 frame. Percent of seedlings exhibiting plant bug damage (i.e. bushy-top) for imidacloprid treated seed averaged 5.5, 4.2, and 3.1% for the 118, 236 and 473ml a.i. treatments, respectively. Damage to the untreated seedlings averaged 10.4%, which was nearly double, to more than triple that of the various seed soaks. Statistical analysis by Waller-Duncan pairwise comparison procedures (SAS Institute 1987) indicated that there were no significant differences amont imidacloprid treatments. However, all three imidacloprid treatments resulted in significantly less bushy-top than control treatments. Despite relatively low damage to controls, these encouraging results prompted an expanded study the following year.

In 1993, imidacloprid solutions corresponding to 0, 236, and 473ml a.i. per 45kg of seed per 189L H_2O were used as 1 hr. seed soak treatments. Seeds were operationally sown in 0.2ha treatment blocks the day after soaking. In addition to the control and imidacloprid seed soaks, a fourth batch of similar but untreated seed was sown and grown in a 0.1ha block under standard operating plant bug protection procedures, consisting of 12 weekly applications of esfenvalerate applied at the rate of 424ml/ha. Damage assessments were recorded on July 23, 81 days after sowing. By that time, all significant damage would have probably occurred.

Plant bug pressure was very high in 1993, based on the average damage exhibited by untreated seedlings (66% of seedlings bushy-topped) and the unexpectedly high level of damage to seedlings receiving protection with conventional esfenvalerate sprays (35.7%) (Table 1). Seed soaks of both 236 and 473ml imidacloprid treatments resulted in significantly lower levels of plant bug damage when compared to controls and standard esfenvalerate treatments (Table 1). Average percent damage in the 236ml treatment samples was 75% lower than that of untreated seedlings and 53% lower than seedlings treated with esfenvalerate sprays. The 473ml treatment samples exhibited a mean low of only 11.0% damage (Table 1); which was 83%, 69%, and 34% less than the damage levels occurring in the control, esfenvalerate and 236ml treatments, respectively. These results indicated that a 1-hr. seed soak of either the 236 or 473ml imidacloprid treatment provided more than two- and threefold greater protection against plant bug damage, respectively, than current management practices.

Other related assessments with regard to the similarities and differences among treatments indicated that there were no significant differences in the number of seedlings per unit area among insecticide treatments and controls (Table 1). However, a significant inverse relationship was exhibited between the mean number of seedlings per unit area and the corresponding incidence of bushy-top in living seedlings ($r^2=0.97$; $P<0.05$). This resulting relationship, where more seedlings per unit area occurred as protection against plant bugs increased (Table 1), suggested that additional plant bug damage may have occurred in the form of early seedling mortality. If plant bugs caused early seedling mortality, the mean trend exhibited for seedlings per unit area by treatment, points towards an additional measure of the effectiveness of imidacloprid seed soaks over esfenvalerate sprays, and esfenvalerate treatment over no control.

Seedling quality was evaluated based on height measurements of sample seedlings in treatment blocks at lifting. Unfortunately, due to poor communication, some treatment blocks were inadvertently lifted prior to measurements, including all of the control trees. Of those seedlings that remained, the highest application rate of imidacloprid seed soak treatments (473ml) resulted in seedlings that were significantly smaller than the 236ml and esfenvalerate treated seedlings (Table 1). Whether or not this approximately 1cm difference or reduction in seedling height was due to the imidacloprid or an indirect result of the protection it affords (increased seedling survival resulting in a higher density of seedlings, leading to greater competition among seedlings, resulting in smaller trees), or has any biologically meaningful ramifications with regard to outplanting success, are unknown.

The significantly improved protection against plant bugs demonstrated with 236 or 473ml imidacloprid seed soak treatments over esfenvalerate sprays, warranted consideration of the potential costs of this alternative approach. Costs of the amount of chemical necessary to conduct the highest imidacloprid treatment (473ml a.i./45kg seed/189L H_20) was $107/ha, based on the manufacturer's projected price for the material. Actual costs of the imidacloprid treatments could not be determined because the product had yet to be registered and was not commercially available. However, at $107/ha the imidacloprid treatment was considerably less expensive than the $154/ha it cost for esfenvalerate insecticide necessary to conduct 12 weekly applications. Imidacloprid seek soak treatments would also provide additional savings by eliminating the costs of labor, fuel, equipment and time associated with weekly spraying.

The desirable attributes inherent with imidacloprid seed soak treatments, coupled with more effective plant bug protection at a lower cost than esfenvalerate sprays, offers a promising alternative to current IPM strategies against plant bugs in conifer nurseries. Imidacloprid is currently registered and labeled for turf and ornamental uses against a wide variety of insect pests, under the trade name Merit®. Merit® is available in water soluble packets, as a wettable powder, or in a granular formulation.

Table 1. Results of 1-hr. imidacloprid seed soak trials to control plant bug injury on sand pine in 1993 at the Andrews' Nursery, Chiefland, Florida.

		Date of Evaluation			
		July 23		December 22	
Treatment[1]	Number Samples[2]	Stocking Trees/4 sq. ft. X±SD[3]	% Bushy Top X±SD[3]	Reps. (Samples) X±SD[3]	Seedling Height (cm)
Control	5	91±15a	67±13a	---[4]	---[4]
Esfenvalerate	3	103±5a	35±5b	3(105)	17±3a
Imidacloprid-236	5	106±5a	17±9c	1(35)	17±3a
Imidacloprid-473	5	111±15a	11±5c	5(175)	16±3b

[1]Control = no insecticides; esfenvalerate=12 weekly applications, each at 424ml/ha; Imidacloprid-236=236ml (a.i.) imidacloprid/45kg seed/189L water; Imidacloprid-473=473ml (a.i.) imidacloprid/45kg seed/189L water.
[2]Each sample equals all seedlings withing a randomly placed 0.37m² frame.
[3]Mean + standard deviation; within each column, different letters indicate significant differences using Waller-Duncan pairwise comparison procedures (SAS Institute 1987).
[4]Data not taken.

WHITE GRUBS

White grubs, the common term referring to larvae of any of several genera and species of Scarabaeidae known as May or June beetles, have been recognized as pests of forest tree seedling nurseries for decades (Speers and Schmiege 1967). In southern forest nurseries, the principle genera that cause seedling damage in nurseries are *Phyllophaga* and *Polyphylla* (Dixon and Foltz 1984). These soil-dwelling grubs may require 1-3 years for development, during which time they usually feed on roots of weeds, grasses, vegetable or cover crops and other organic matter. However, they also feed on tender roots of all types of coniferous and hardwood seedlings in nurseries. Damage to seedling crops is evidenced by severed or girdled tap roots, missing lateral roots and gouges in large roots. Damaged seedlings are easily pulled from the soil (Cordell et al. 1989). Injury may become evident by the browning and death of patches of seedlings, beginning as early as June (Bacon and South 1989).

The larvae are characteristically recognized by their brownish head, creamy white body, translucent hind end, C-shape, well developed legs and mandibles, and relatively large size (about 2.5cm). Severe losses may be expected when these larvae number 1 or more per 929cm² of planting surface (Cordell et al. 1989). White grub problems are typically most severe on light, sandy soils which are typical of Florida nurseries, and newly turned seedbeds (Dixon et al. 1991; Spears and Schmiege 1967).

Current IPM recommendations against white grubs include clean cultivation practices and repeated discing of planting ground in the fall and winter prior to spring sowing, scouting for grubs throughout seedbed preparation, regular monitoring for grubs and seedling injury in nursery beds beginning in midsummer, preventive applications of granular insecticides or soil fumigation, and remedial spot treatment with insecticides to suppress damaging populations (Bacon and South 1989; Dixon et al. 1991; Sutherland, personal communication).

Nursery managers that rely solely on soil fumigation to control damaging populations of white grubs, and fail to conduct early detection monitoring, can suffer serious losses. Overwintering and actively feeding larvae in the spring and summer can reside at or move deeper in soil below effective fumigation zones. Fall or spring fumigation also fails to prevent annual egg loads from being deposited and developing in soil every year during the summer. Widespread use of soil stabilizers or sealants on sandy soils in southern nurseries, and the frequent irrigation required, delays onset of aboveground symptoms of grub injury until long after serious damage and losses have occurred. Scouting for larval activity is therefore very important.

One Florida nursery recently experienced a significant white grub problem due to lack of anticipating problems following fumigation. The affected nursery had been fumigated with Basimid® at the rate of 336kg/ha in March 1993 and was followed with a successful crop of slash pine, harvested in the winter of 1993-94. The area was again fumigated with Basimid® as before on March 18, 1994 and sowed with slash pine seed in April. Seedlings had been root pruned on approximately July 1st and exhibited no noticeable problems. With no apparent above ground symptoms evident by the fourth week of August, sample seedlings were pulled to determine required setting of a second undercutting. In the process it was noted that many seedling root systems were virtually devoured. A systematic survey revealed 1.42ha of affected seedlings and associated high grub populations of greater than 1 per 929cm^2. Seedlings on 0.4ha, where as high as 11 grub larvae per 929cm^2 occurred, were so severely damaged that trees could not be salvaged. Affected seedlings were plowed under, resulting in a loss of approximately 689,000 seedlings worth $20,670. The remaining and salvageable 1.02ha were sprayed with two applications of chlorpyrifos, at the rate of 9.35L/ha, after thoroughly watering beds before and after insecticide treatments to bring grubs to the surface and move insecticide into the soil. No grubs or new damage occurred after treatment.

There are well over 1,400 species of Scarabeidae documented in the United States including 54 species of *Phyllophaga* occurring in Florida. Unfortunately, there are no known definitive larval descriptions and little biological information is known for most of these species. Because many of these insects are not plant pests, sighting of grub larvae even at high population densities is not reason enough to immediately treat nursery beds with insecticides. For example, during a recent research project (Barnard et al., unpublished), routine sampling of slash pine nursery beds revealed an abundant population of white grubs in many treatment blocks. Larvae were tentatively identified as *Hybosorus illigeri* Reiche and *Psammodius malkini* Cartwright, two scarabs for which little biological information is known. These species were not known pests of pine in Florida nurseries and there was no apparent root damage associated with their occurrence. Therefore, affected areas were not treated and insect populations were closely monitored. Fortunately, no observable damage ever materialized and the costs of treating with insecticides were avoided. Therefore, grub larvae should be identified by expert taxonomists and feeding damage should be monitored before initiating control treatments.

Another example of where IPM techniques for white grubs produced substantial savings over traditional control approaches occurred when a nusery expanded into two areas that had previously been Bahia grass (*Paspalum notatum* Flugge) pasture. Conversion of agricultural crop or pasture land into pine nurseries has historically resulted in severe white grub damage to seedlings (Dixon et al. 1991). Areas of freshly turned sod have traditionally been fumigated prior to growing pine crops. Initial grub surveys in Bahia grass areas revealed low larval populations. This, in addition to cost considerations and the desire to limit chemical use, prompted decisions not to fumigate, but rather to culturally prepare seedbeds in ways destructive to grubs. These procedures involved repeated bottom plowing, chisel plowing, discing, and clean cultivation; yielding fewer and fewer grubs throughout the summer. Longleaf pine (*P. palustris* Mill.) seed were sown in October, grown and harvested without any noticeable grub damage or need for treatment. Careful monitoring and using cultural instead of chemical control methods resulted in substantial savings to nursery growers.

SPIDER MITES

Spider mites (Acari: Tetranychidae) in the genera *Oligonychus* and *Tetranychus* can cause damage to southern pine nursery stock. Severe spider mite infestations produce a stippling, mottling and curling effect of foliage, causing seedlings to appear washed out, yellow or bronze. Spider mite populations can rapidly increase to damaging levels because of extremely short generation times (4-12 days). Outbreaks in nurseries typically develop between late summer and early winter, during hot, dry weather. Severe damage rarely causes seedling mortality but may weaken trees, predisposing them to other mortality factors or leading to reduced growth and poor field survival (Bacon and South 1989; Cordell et al. 1989; Dixon et al. 1991).

Prior to 1987, the standard operating procedure at one Florida forest nursery was to wait until damage appeared and then spot treat affected areas with repeated malathion sprays. This only resulted in chasing spider mite problems every year rather than solving problems. Damaged seedlings would typically begin to appear in November and reoccur in other untreated areas of the nursery throughout the remainder of the growing season. Spider mite damage was continuously monitored and expensive insectide treatments were usually required. The relative ineffectiveness of preventing or controlling spider mite damage prompted a change in tactics during 1987. Prior to any noticeable injury, the entire

nursery crop was treated with an application of the miticide dicofol around Nov. 1, followed by a second application 2-3 weeks later. The miticide was applied with a surfactant at a rate of 2.34L/ha using a high-pressure sprayer. Spider mite problems have failed to reoccur since these miticide applications have become routine.

Concerns over costs, safety, side-effects (e.g., toxicity to predaceous mites) and possible development of insecticide-resistant spider mite populations of dicofol treatments generated efforts to find more suitable control alternatives. The relatively recent discovery, introduction, use and reported effectiveness of abamectin (avermectin B_1) against spider mites on woody ornamentals, offered another IPM alternative. Abamectin has several advantages over conventional pesticides available for spider mite control. These include a potent and naturally derived mode of action making it effective against insect strains resistant to other products, long residual activity due to penetration and persistence within foliage, relatively lower impact on beneficial organisms and potential for environmental contamination, and wide plant applicability and margin of safety for workers (Merck AgVet Division 1992). To test abamectin's ability to control spider mites on pine seedlings, four, 1.24ha blocks of slash pine nursery beds were left untreated during the nurserywide dicofol applications in November, 1993. Within one of these 1.24ha blocks, an abundant population of spider mites (*Oligonychus milleri* McGregor) became evident when visual damage symptoms were detected in January, 1994. Seedling examinations at the time revealed presence of all mite life stages. The next day, the entire block was treated with a spray mixture corresponding to 1.17L of abamectin/ha (maximum recommended application rate) in about 208L of H_20, requiring 3 passes for total delivery. Four days following treatment there was still no noticeable decline in mite numbers nor their activity and a second application was performed. This second application was at the rate of 0.585L abamectin/ha in 89L of H_20, requiring 1 pass. Five days later, a noticeable reduction in mite activity was observed, but substantial populations of active immatures and adults and an abundant supply of unhatched eggs persisted. After a week, increasing mite activity and associated damage in the test block necessitated an application of dicofol mixed with crop oil, following which no spider mite activity was ever observed. Abamectin was ineffective in controlling spider mites in this one example. However, because of its potential as a desired alternative for control, additional efficacy tests are planned.

LESSER CORNSTALK BORER

Larvae of the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller), are most commonly associated with injury of a wide variety of grass and legume crops throughout the southeastern United States, but can also cause substantial damage to several tree seedling species in nurseries. Nearly one million pine, baldcypress (*Taxodium distichum* (L.) Rich.), southern red-cedar (*Juniperus silicicola* [Small] Bailey), and dogwood (*Cornus florida* L.) seedlings were killed by lesser cornstalk borer in a Florida nursery during 1981 (Dixon 1982b). Seedling damage and mortality occurs from belowground larval feeding, resulting in partial to complete girdling of the main stem and root scarring. Gall-like swellings are typically formed at feeding sites (Cordell et al. 1989; Dixon 1982a, 1982b).

Factors favoring borer infestations include millet, corn and soybean cover crops, mulches and organic amendments (e.g., peanut shells), sandy soils and hot, dry weather. Control recommendations include avoiding susceptible cover crops and mulches, rotating with nonsusceptible cover crops, practicing general sanitation measures such as clean cultivation, and late fall plowing. Preventative treatments with granular insecticides when planting cover crops and treating seedlings when damage occurs with soil drenches of carbaryl or chlorpyrifos are also effective (Bacon and South 1989; Dixon 1989). Because of protective silk tubes formed by larvae in the soil and multiple (2-4) generations exhibited by the insect, several insecticide applications may be required.

Lesser cornstalk borer-caused damage and losses are commonly reported in Florida forest nurseries despite treatments. More effective control measures for this pest are desired, despite maximum annual production losses of less than 2% (Dixon 1982b). However, no known attempts have been made to improve existing strategies. One relatively simple way of improving effectiveness and reducing costs of current controls would be accurate timing of insecticide applications prior to initial detection of damage symptoms, when the first generation of larvae are present. Commercially-available pheromone traps for this insect could be used to monitor emergence of moths from overwintering populations late in the spring. Insecticide treatments could then be timed to coincide with susceptible larval stages of the first generation. This approach would improve existing methods by targeting pest populations before intial damage occurs, targeting the first generation, subsequently limiting those that will follow, and more closely matching insecticide applica-

tion with susceptible larval stages for more thorough control.

REGENERATION WEEVILS

The pales weevil (*Hylobius pales* [Herbst]) and pitch-eating weevil (*Pachylobius picivorus* [Germar]) normally do not cause damage in pine nurseries (Bacon and South 1989), but can cause serious damage to outplanted seedlings. First-year seedlings in the field are often killed by adult weevils when planted during November-February on sites where pines have been killed or harvested in the previous 5 months. Conventional methods of preventing losses have been to either delay planting for one year or treat seedlings with insecticides (carbofuron and phosmet) before or after lifting and prior to planting (Nord et al. 1982). Chlorpyrifos applications can also prevent damage before and after outplanting (Bacon and South 1989).

An alternative to conventional control procedures was recently required in a Florida nursery. Sixteen hectares of state forest land which had been harvested in 1994, and scheduled for reforestation in the winter of 1994-95, unfortunately adjoined an 445ha area of forest severely damaged by a wildfire. Salvage and thinning in the burned area were not completed until November 1995, creating a high risk of regeneration weevil damage on the adjacent 16ha planting site. Delaying planting for one year was undesirable because site preparation was already completed. Conventional insecticide treatments of nursery seedlings using dips before planting were unacceptable due to associated costs and safety risks to inexperienced workers. However, a recently registered chemical to control regeneration weevils for field-grown conifers (Pounce® 3.2 EC (= permethrin) was a desirable alternative if it could be effectively applied in the nursery. Based on effectiveness of 4-5 years of operational nursery treatment of seedlings in Virginia with this chemical 2-3 days prior to lifting (Tigner, personal communication), treatments were planned in Florida. An 585ml/ha rate of Pounce® 3.2 EC in 132L of H_2O was applied to longleaf pine seedlings with a tractor mounted, overhead spray rig, 3 days prior to lifting. Seedlings were then planted and have subsequently exhibited no weevil damage.

Effective dosage rates need to be determined using controlled experiments with untreated seedlings for comparisons.

LITERATURE CITED

Bacon, C.G. and D.B. South. 1989. Chemicals for control of common insect and mite pests in southern pine nurseries. Southern Journal of Applied Forestry 13:112-116.

Bryan, H. 1989. Control of the tarnished plant bug at the Carters Nursery. Tree Planters' Notes 40(4):30-33.

Cordell, C.E., R.A. Anderson, W.H. Hoffard, T.D. Landis, R.S. Smith, Jr., and H.V. Toko (eds.). 1989. Forest Nursery Pests. USDA Forest Service. Agricultural Handbook No. 680. 184p.

Dixon, W.N. 1982a. Lesser cornstalk borer, *Elasmopalus lignosellus* (Zeller), (Lepidoptera: Pyralidae). Florida Department of Agriculture and Consumer Services, Division of Plant Industry. Entomology Circular No. 236. 2p.

Dixon, W.N. 1982b. Lesser cornstalk borer damage to forest nursery seedlings in Florida. Tree Planters' Notes 33(4):37-39.

Dixon, W.N. 1989. The tarnished plant bug, *Lygus lineolaris* (Palisat de Beauvois) in conifer nurseries (Heteroptera: Miridae). Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Entomology Circular No. 320. 2p.

Dixon, W.N., E.L. Barnard, C.W. Fatzinger, and T. Miller. 1991. Insect and disease management. *In*: Duryea, M. L. and P.M. Dougherty (eds.), Forest Regeneration Manual. Klower Academic Publishers. pp. 350-390.

Dixon, W.N. and J.L. Foltz. 1984. Integrated pest management in nurseries - insects and mites. *In*: Branham, S. J. and G. D. Hertel (eds.). Proceedings Integrated Forest Pest Management Symposium, University of Georgia, Center for Continuing Education, Athens, GA. pp. 226-237.

Merck AgVet Division. 1992. Avid®technical profile. Merck AgVet Division, Rahway, NJ., USA. 11p.

Mullins, J.W. 1993. Imidacloprid: a new nitroguanidine insecticide. *In*: Duke, S. O., J. J. Menn and J. R. Plummer (eds). Pest Control with Enhanced Environmental Safety. American Chemical Society Symposium Series No. 524. American Chemical Society, Washington D.C. pp. 183-198.

Nord, J.C., J.H. Ghent, H.A. Thomas and G.A. Doggett. 1982. Control of pales and pitch-eating weevils in the south. USDA Forest Service, Southern Region, Forest Report SA-F 21. 24 p.

SAS Institute. 1987. SAS/STAT Guide for Personal Computers, Version 6 Edition. Cary, NC. SAS Institute, Inc. 1028p.

South, D.B. 1986. The "tarnished plant bug" can cause loblolly pine seedlings to be "bushy-topped". Auburn University Southern Forest Nursery Management Cooperative Newsletter No. 27. 6p.

South, D.B. 1991. *Lygus* bugs: a worldwide problem in conifer nurseries. *In*: Sutherland, J. R. and S.G. Glover (eds.). Proceedings of the First Meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada, Pacific and Yukon Reg., Information Report BC-X-331. pp. 215-222.

South, D.B. and J.B. Zwolinski. 1993. A new insect pest of pine nurseries identified. Highlights of Agricultural Research. Alabama Agricultural Experiment Station, Auburn University, Alabama 40(1):16.

South, D.B. and J.B. Zwolinski. 1996. Chemicals used in southern forest nurseries. Southern Journal of Applied Forestry 20(3):127-135.

South, D.B., J.B. Zwolinski and H.W. Bryan. 1993. *Taylorilygus pallidulus* (Blanchard): a potential pest of pine seedlings. Tree Planters' Notes 44(2):63-67.

Speers, C.F. and D.C. Schmiege. 1967. White grubs in forest tree nurseries and plantations. USDA Forest Service. Forest Pest Leaflet No. 63. 4p.

United States Department of Agriculture. 1985. Insects of eastern forests. USDA Forest Service. Miscellaneous Publication 1426. 608p.

FOREST NURSERY SCARABS IN NORTHWEST CHINA[1]

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ABSTRACT

Northwest China comprises about 30% of the country's land area and is characterized by a dry climate and sparse forests. Seedling production and afforestation in this area is difficult. Severe seedling losses are often sustained in forest nurseries in Northwest China as a result of scarabs which cause seedlings to wilt and die. Eighteen species of scarabs are important pests in these nurseries. These are *Anomala corpulenta*, *Chioneosoma reitteri*, *Holotrichia diomphalia*, *H. parallela*, *H. titanis*, *H. trichophora*, *Maladera orientalis*, *M. verticalis*, *Melolontha hippocastani*, *M. tarimensis*, *Metabolus flavescens*, *Oxycetonia jucunda*, *Polyphylla gracilicornis*, *P. laticolis*, *Popillia quadriguttata*, *Potosia brevitarsis*, *Proagopertha lucidula* and *Trematodes grandis*. These insects have different biological habits and characteristics and are widely distributed under varying ecological conditions. Prevention and control measures include insecticide applications, planting resistant plant cultivars, and installing ultraviolet lamps to trap nocturnal scarab adults. Attention is also given to utilization of natural enemies when possible; potential of sex pheromones is currently being studied.

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STUDIES ON SELECTION OF MAJOR PESTS TO POPLAR SEEDLINGS AND POPLAR SEEDLING PEST RESISTANCE[1]

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ABSTRACT

This paper was based on experiments evaluating major pest preferences for randomly-arranged seedlings from different *Populus* taxonomic sections located within selected forest nurseries in China. Seedlings were grown in open-air nurseries and subjected to natural insect pest infestations; data were taken on extent of damage to various poplar species and hybrids. The small poplar borer (*Saperda populnea*) commonly infested poplar seedlings in sections *Aigeiros* Duby and *Tacamakaca* Spach. and their hybrids; seedlings in section *Leuce* Duby were unharmed, whereas those of *P. hopeiensis* Hu and Chow were slightly damaged by this insect species. The poplar clearwing (*Paranthrene tabaniformis*) caused most damage to *P. tomentosa* Carr and *P. xeramericana* cv. sacrau 79. The clearwing was also damaging to all seedlings in section *Leuce*, but less harmful to seedlings in section *Aigeiros* and their hybrids. Poplar leaf beetle (*Chrysomela populus*) caused damage to most all species and hybrids examined. However, seedlings in section *Leuce* were usually less damaged than those in section *Aigeiros*. The willow leaf beetle (*Plagiodera vesicolora*), an introduced pest in China, caused damage to almost all seedling species and hybrids examined. White poplar leafminers (*Leucoptera susinellais*) were likewise damaging to most poplar genotypes. The palisade sawfly (*Stauronematus compressicornis*) caused slight damage to some seedlings in sections *Leuce* and *Aigeiros* and their hybrids. The poplar sawfly (*Pristiphora conjugata*) damaged a few seedlings in the section *Aigeiros* and their hybrids. The final insect detected was the one-marked green moth (*Earias pupillana*), which caused damage only to seedlings in section *Leuce* with "hairy" tops.

[1] Paper presented at the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). Orlando-Gainesville, FL., USA. May 19-24, 1996. Only the abstract is available; completed paper was not provided for proceedings.

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DISEASES OF SEEDLINGS IN FOREST TREE NURSERIES IN FLORIDA: HISTORY AND STATUS[1]

E.L. Barnard[2]

ABSTRACT

Important diseases in Florida's forest tree nurseries have included fusiform rust, a *Rhizoctonia* blight of longleaf pine, *Phytophthora* root rot of sand pine, charcoal root rot, pitch canker disease of pines, stem cankers on container-grown eucalypts, brown spot needle blight of longleaf pine and *Phomopsis* blight of redcedar. Other diseases of lesser overall impact have included tip blights of pine, a root knot nematode-charcoal root rot complex on dogwood, a blight of redcedar caused by *Sclerotium rolfsii*, *Pythium* and *Phytophthora* root rots of dogwood and oaks, and a blight of container-grown longleaf pine caused by *Calonectria kyotensis*. Other diseases have included miscellaneous nutrient deficiencies and fumigation-induced mycorrhizal deficiencies in vesicular-arbuscular hosts. Nursery-to-field carry-over of nursery-initiated infection has been a focus of attention for a number of host- pathogen combinations. This paper provides a historical overview and current status report for the state's more important forest nursery diseases. Control stategies are also discussed.

INTRODUCTION

Production of seedlings in forest tree nurseries in Florida began in the late 1920's. By the mid-1930's, pine seedling production had arrived in earnest with statewide annual production totaling approximately 14,000,000. By the 1940's statewide annual production had reached 100,000,000 and annual production peaked in the late 1980's at approximately 195,000,000 seedlings. These figures are inclusive for a constantly changing mix of state-owned, industrial, and private nurseries.

Although 1-0 bare-root pines have accounted for the overwhelming majority of seedlings produced, production has not been limited to pines. Over the years, a number of bare-root nurseries have produced various hardwood species, baldcypress [*Taxodium distichum* (L.) Rich.] and redcedar (*Juniperus virginiana* L.). Also, some nurseries produce crops of containerized seedlings including some ornamental species, but more particularly, and in larger quantities, species of *Pinus* and *Eucalyptus* for afforestation or reforestation.

This paper provides an overview of the more important diseases that have historically impacted forest tree seedling quality and production in Florida. Specifics with respect to pathogen biology are minimized because this information is largely available in the phytopathological literature. Instead, emphasis is placed upon Florida's forest nursery disease history, experience, management and current situations.

HISTORICAL SYNOPSIS

Common and problematic diseases in the "early days" presumably included episodes of damping-off, fusiform rust [caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Hedgc. & N. Hunt) Burdsall & G. Snow], and possibly parasitism of roots by one or more species of plant-parasitic nematodes. In the late 1950's and 1960's black root rot [caused by a "complex" of *Macrophomina phaseolina* (Tassi) Goid., *Fusarium* spp., and plant parasitic nematodes] and charcoal root rot (caused predominantly, if not strictly by *M. phaseolina*) emerged as major problems (Hodges 1962; Seymour 1969). By the mid-1970's, charcoal root rot was considered the single most serious disease impacting Florida's pine seedling nurseries. Losses to this disease in 1976 were estimated to be at least 20 million seedlings, including an estimated 16.5 million seedlings (approx. value = $148,000.00) quarantined in one nursery alone due to the disease (Seymour and Cordell 1979). This episode resulted in the creation and staffing of the state's first and only forest pathologist position.

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Since 1978, attention has been given to a wide variety of seedling diseases, some of which represent major threats to seedling production and others which are only sporadic in their occurrence or ephemeral in nature and cause little impact. Among the more important and impact-producing diseases addressed are fusiform rust, a "*Rhizoctonia* blight" of longleaf pine (*Pinus palustris* Mill.), *Phytophthora* root rot of sand pine [*P. clausa* (Chapm.) Vasey] caused by *P. cinnamomi* Rands., charcoal root rot, pitch canker disease of pines, stem cankers on container-grown *Eucalyptus* spp., brown spot needle blight of longleaf pine, and *Phomopsis* blight of redcedar. Episodic nursery diseases of less overall consequence have included root diseases of sand pine caused by *P. nicotianae* B. de Haan var. *parasitica* (Dastur) Waterh., pine "tip blights", a root knot nematode - charcoal root rot complex on dogwood (*Cornus florida* L.), a blight of redcedar caused by *Sclerotium rolfsii* Sacc. (teleomorph; *Athelia rolfsii* Tu and Kimbrough), *Pythium* and *Phytophthora* root rots of dogwood (*Cornus florida* L.) and oaks (*Quercus* spp.), and a previously unreported blight of container-grown longleaf pine caused by *Calonectria kyotensis* Terashita.

Other "diseases" which have been documented and addressed include miscellaneous nutrient deficiencies and fumigation-induced mycorrhizal deficiencies in a variety of vesicular-arbuscular hosts. Ectomycorrhizal deficiencies, for the most part, have not been problems due to rapid reinfestation of fumigated nursery soils by the common basidiomycetous symbionts *Thelephora terrestris* Ehrh.:Fr., *Rhizopogon nigrescens* Coker & Couch, and in certain situations, *Pisolithus tinctorius* (Pers.) Coker & Couch.

Of particular concern with respect to many of the nursery diseases mentioned above has been the potential for nursery-to-field carry-over and latent disease development subsequent to outplanting. Specifically, focus has been directed upon identifying those pathogens that might be associated with such diseases and evaluating their damage potential (Barnard 1984, 1994; Barnard et al. 1985b).

THE CURRENT SITUATION

The following are brief status reports on Florida's more important and interesting forest nursery diseases. Approaches to disease management and control are discussed.

Damping-off

Major damping-off problems in Florida's forest tree nurseries are infrequent. Several factors may account for this desirable situation. Among such factors are: (1) the generally sandy, well-drained nature of Florida's bare-root nursery soils, (2) environmental conditions conducive to rapid seed germination, (3) routine utilization of high-quality, pathogen-free seed, (4) seed treatments such as thiram, and (5) maintenance of innocuous populations of soilborne pathogens through intelligent crop rotations and periodic soil fumigation with methyl bromide or other soil sterilants. Damping-off in containerized nursery operations is also minimized through the use of pre-sterilized or pathogen-free potting mixes.

In cases where problematic levels of damping-off have occurred and have been evaluated, common soilborne damping-off pathogens including species of *Pythium*, *Rhizoctonia* (or *Rhizoctonia*-like fungi), and *Fusarium* are often detected. In some cases, the charcoal root rot pathogen, *M. phaseolina*, has been implicated, but this is not particularly common. Interestingly absent from the pathogen mix have been species of *Phytophthora*.

Fusiform Rust

Although this is considered the most serious disease impacting southern pine forestry (especially the intensive culture of *Pinus taeda* L. and *P. elliottii* Engelm.) in both nurseries and plantations, surprisingly little is actually known regarding the annual impact of fusiform rust in Florida forest nurseries. In part, this information gap is due to (1) the traditionally excellent fungicidal control programs employed by nursery managers (formerly ferbam but currently triadimefon-based) and (2) the lack of unprotected impact assessment or monitoring plots in operational nurseries (nursery managers spray all seedlings; no unsprayed seedlings are evaluated). However, the potential for catastrophic losses was dramatically emphasized in one Florida nursery in 1979 where disease incidence was so high (approximately 75-80% of seedling stems infected across one nursery block) that approximately 7.5 million seedlings valued at approximately $97,000 were destroyed. This incident occurred despite repeated prophylactic applicatiosn of the contact fungicide ferbam. Since the early 1980's and the introduction of triadimefon fungicide for control of fusiform rust

in forest nurseries (Carey and Kelley 1993; Kelley 1985; Kelley and Runion 1991; Powers 1984; Rowan 1982; Snow et al. 1979), losses have been minimal.

Charcoal Root Rot

At one time clearly considered among Florida's most serious forest nursery diseases, charcoal root rot has diminished in severity in recent years. Nursery managers have, for the most part, adopted management strategies which minimize the potential for serious disease losses. Such strategies include avoidance of certain cover crops (such as legumes) which enhance buildup of soilborne *M. phaseolina* populations, minimizing water stress to seedlings during high temperature periods or following root pruning or undercutting, and soil fumigation.

Historically, charcoal root rot has been considered a serious pine seedling disease (Barnard and Gilly 1986; Hodges 1962; Rowan 1971; Seymour 1969; Seymour and Cordell 1979). However, *M. phaseolina* is probably at best an opportunistic pathogen, responsive to soil fertility levels, irrigation, soil temperatures, plant stress and senescence (Dhingra and Sinclair 1978; Hodges 1962; McDonald and Mehan 1984; Reuveni and Madar 1985; Watkins 1981; Wylie 1989). Indeed, this author has witnessed charcoal root rot scenarios in pine nurseries only in association with cultural practices of conditions which could be viewed as suspect and where seedlings have been subjected to unnecessary stress (untimely and purposely-induced water stress to harden-off seedlings, root pruned seedlings subjected to excessive water stress, etc.). Accordingly, the question may be asked, "Is this a biotic disease caused by a serious pathogen, or a biotic response to ill-advised or accidental nursery management or mismanagement?"

For some time, pine seedling outplant failures, usually on former agricultural lands in Florida, were often blamed on nursery-originated *M. phaseolina* infections because this organism was sometimes detected on seedlings that died following outplanting. This "nursery linkage," unfortunately, was often more a function of nursery reputation and a presumed understanding of the biology of the pathogen than hard core evidence *per se*. Such interpretations enhanced the belief that *M. phaseolina* was a serious nursery pathogen. However, recent evidence reinforces the relatively cosmopolitan distribution of *M. phaseolina*, especially in Florida agricultural soils, and the organism's role as a stress-responsive secondary colonizer (Barnard et al. 1995; Barnard et al. 1994a). Further, attempts to simulate nursery-to-field carry-over of *M. phaseolina* on roots of asymptomatic seedlings suggest that, although such transport is possible, it may not occur at high frequency or be a management concern (Barnard 1994).

Phytophthora Root Rots of Sand Pine

Sand pine is a relatively small, short-lived southern yellow pine native to Florida's sandhills. This species makes up a small component of Florida's commercial forest mix, but is still produced in some forest nurseries. It is used primarily for Christmas trees and planted on sites unsuitable for the more popular slash and loblolly pines.

Historically, sand pine has been plagued by serious root disease problems including both nursery and plantation infections by *Phytophthora cinnamomi* (Barnard 1988; Barnard et al. 1985; Ross and Marx 1972). *P. cinnamomi* infections are serious on sand pines which are apparently innately susceptible to root disease infections in general. Infected trees usually succumb at an early age. Although differences in susceptibility between the Choctawhatchee variety (*P. clausa* var. *immuginata* Ward), native to Florida's western panhandle, and the Ocala variety (*P. clausa* var. *calusa* Ward), native to peninsular Florida, have been reported (Barnard et al. 1993; Ross and Marx 1972), such differences are inconsistent and nursery managers, foresters and landowners cannot rely upon apparent resistance to avoid losses. Evidence is strong that many *P. cinnamomi* disease scenarios in sand pine plantations are a function of nursery-to-field carry-over of the pathogen on the roots of outplanted seedlings (Barnard et al. 1985a, 1985b).

Of lesser importance, and perhaps only significant as mycological or pathological curiosities, have been ephemeral root rots and associated mortality of sand pine seedlings caused by *P. nicotianae* var. *parasitica*. Three episodes of this host-pathogen association have been documented in Florida forest nurseries; two in bare-root nurseries and one in a containerized nursery. In each case, seedling mortality was rapid and occurred in the heat of Florida's mid-growing season (July-September) in water-saturated or puddled soils. Disease spred in affected seedbeds or containers was minimal and was apparently arrested with the onset of improved soil drainage. *P. nicotianae* var. *parasitica* has not been detected on planted or naturally established sand pines in the field.

Conventional wisdom might suggest that *Phytophthora* root rots of sand pine could be controlled by soil fumigation with methyl bromide. However, every

case of such infections I know of in Florida forest nurseries has occurred in fumigated soils. Indeed, in the one Florida nursery with a documented resident population of *P. cinnamomi* (Barnard et al. 1985b), there was evidence that the pathogen survived in fumigated seedbeds at depths beneath the influence of routine fumigation (Barnard, unpublished). Also, the high clay content of soil at this nursery may have restricted efficacy of soil fumigation (pathogens surviving in soil clods, etc.). In the case of *P. nicotianae* var. *parasitica*, it is possible that pathogen inoculum may be introduced via irrigation water, or it too may survive beneath the zone of fumigation influence.

Phytophthora root diseases in Florida forest nurseries are currently inconsequential. The only nursery with a history of *P. cinnamomi* has not produced sand pine for nearly a decade and in fact is now closed. The remaining nurseries are apparently *P. cinnamomi*-free, contain sandy, adequately drained seedbeds, and are well managed. Whether or not soil fumigation, routinely practiced at most Florida nurseries, is important in controlling *Phytopthora* root diseases is unknown.

Phytophthora and Pythium Root Rots of Hardwoods

Although not a major problem, root rots (as opposed to damping-off) of hardwood species caused by *Phytophthora cinnamomi* and *Pythium* spp. have occurred in Florida forest nurseries. In every case I know of, infections have occurred in methyl bromide-fumigated seedbeds in areas where shallow clay layers impeded soil drainage. Infections started in lateral or tap roots penetrating soil beneath the influence of the soil fumigant. Hosts affected have included *Cornus florida*, and *Quercus* spp.

Rhizoctonia Blight of Longleaf Pine

This disease, sometimes referred to as "sand splash" (Davis 1941), has caused serious losses of longleaf pine seedlings in both bare-root and containerized nurseries in Florida. Formerly attributed to *Rhizoctonia solani* Kuhn (Barnard 1979), we now know the disease is caused primarily by a binucleate *R. solani* - like fungus belonging to anastomosis group 3 (CAG 3) of the genus *Ceratóbasidium* (English et al. 1986). On one occasion, a foliage blight apparently caused by the same organism, was documented on loblolly pine in a Florida nursery. However, primary occurrence remains on longleaf pine due largely to the "grass stage" seedling habit of the latter.

Fungicides have proven relatively unsatisfactory to control this disease in Florida. Nursery managers have mostly relied on cultural approaches to minimize losses. Fall sowing of longleaf pine seed, together with carefully selected, well-timed, and repeated seedbed mulches generally provide adequate disease control (Barnard et al. 1994b; Davis 1941; Gilly et al. 1985). Soil fumigation appears to give some measure of control, but pathogen inoculum sources are not well defined.

Pitch Canker Disease of Pines

Fusarium subglutinans (Wollenw. & Reinking) Nelson, Toussoun & Marasas causes a distinctive canker and dieback disease on a variety of *Pinus* spp. (Barnard and Blakeslee 1980; Blakeslee et al. 1980; Dwinell et al. 1985). This fungus is also capable of infecting pine cones and seed and can cause seedling infections ranging from pre- and post-emergence damping-off to tip-blight, root rots, lethal stem cankers and root collar lesions. Pitch canker was first reported in 1980 in Florida as a new nursery disease of slash pine (Barnard and Blakeslee 1980). Since that time it has appeared periodically in various pine seedling crops. Fortunately, seedling losses to pitch canker infections are typically inconsequential in most crops (< 1-5%), although substantially higher losses (10-15%) have been recorded for certain slash pine crops on a seedlot by seedlot basis (G.M. Blakeslee, personal communication). Slash pine is the primary species affected by *F. subglutinans* in Florida nurseries, but infections also occur on sand and longleaf pines. Fungicides are ineffective for control (G.M. Blakeslee, personal communication). For the most part, disease impact is minimized through use of resistant seed sources, sound seed orchard management and clean seed handling and processing procedures, ensuring pathogen-free, high-quality seed.

Nursery-to-field carry-over of pitch canker infections are of concern to nursery managers and foresters in Florida, primarily because seedling infections are typically lethal. Field observations and preliminary studies (Barnard, unpublished; G.M. Blakeslee, personal communication) provide evidence supportive of a nursery-to-field carry-over linkage, but definitive research is still wanting.

Brown Spot Needle Blight

Brown spot needle blight, caused by *Mycosphaerella dearnessii* Barr [=*Scirrhia acicola* (Dearn.) Siggers], has caused serious losses in longleaf pine seedlings in forest nurseries in Florida's western

panhandle. Production of longleaf pine in these nurseries requires a rigorous fungicidal protection program using primarily chlorothalonil and/or benomyl. Brown spot-free seedlings are necessary for regeneration programs since nursery-initiated infections can intensify under field conditions, resulting in mortality or poor performance. Most of Florida's nursery production of longleaf pines is now located in the state's northern peninsular region where disease pressure is less intense than it is in the panhandle region. Concomitantly, current losses to brown spot infections are minimal, and the need for judicious fungicidal protection has been oblated.

Pine Tip Blights

The periodic occurrence of pine seedling tip blights in Florida's forest nurseries causes concern to nurserymen from time to time. Described as "tip dieback" by Rowan (1982b), these phenomena are apparently caused by a variety of fungi. Rowan (1982b) reported that *Fusarium subglutinans*, *Lasiodiplodia theobromae* Pat. (=*Diplodia gossypina* Cke.) and a *Phomopsis* sp. were all capable of inducing dieback symptoms when artificially inoculated onto the wounded terminals of 1-year-old loblolly pine seedlings. These, and a number of other fungi (some of uncertain taxonomic affinity), including on one occasion *M. phaseolina*, have been associated with tip blights on pines in Florida forest nurseries over the years (Barnard, unpublished). It appears that tip blight diseases are the result of opportunistic infections by a variety of fungi that take advantage of warm, humid environmental conditions and the tender, succulent growing shoots of young pines abundantly provided in forest tree nurseries. Evaluations in Florida (Barnard, unpublished) indicate high levels of tip blight to be associated with nursery locations prone to moisture buildup and/or retention (low spots or depressions in seedbeds) and seedling crops exhibiting particularly rapid development (thus, elongated tender, succulent growing shoots). Field observations have also revealed essentially full recovery of blighted seedlings within 30 days following onset of visual symptoms. Tip blights of this nature appear to be more an ephemeral phenomenon than a disease of consequence.

Cylindrocladium Stem Cankers of Eucalyptus spp.

In the 1970's and early 1980's, the Florida Division of Forestry produced several million containerized *Eucalyptus* seedlings for commercial forestry plantings in south-central peninsular Florida. Peak production reached approximately two million seedlings per year in the late 1970's. *Eucalyptus* production was terminated in 1982-83 due to a series of damaging freezes and changing forestry priorities. However, during the period of *Eucalyptus* production, *Cylindrocladium scoparium* Morgan surfaced as a major forest nursery pathogen in Florida. This organism, already well known as a pathogen of *Eucalyptus*, especially in South America (Barnard 1984), caused major losses in crops of *E. grandis* Hill ex Maid. and *E. robusta* Sm. in a containerized nursery in Highlands County (south-central peninsular Florida) by inducing distinctive and often lethal girdling stem cankers (Barnard 1984). A fungicide trial (Barnard 1984) showed chlorothalonil and benomyl to provide effective control, but emphasis was ultimately placed on changing cultural protocols such as improving container spacing and aeration and reducing irrigation. A field outplant trial (Barnard 1984) demonstrated that the pathogen had little residual effect on seedlings once they were planted onto field sites. Only seedlings with advanced stem cankers in the nursery failed to perform well once outplanted. The pathogen acted aggressively only in nurseries characterized by closely spaced, poorly aerated, and heavily watered seedlings in a warm, humid environment.

Calonectria Blight of Containerized Longleaf Pine

As *Eucalyptus* production subsided in the early 1980's, the Florida Division of Forestry increased production of containerized pine seedlings. Production was initially centered in the Division's Highlands County (south-central Florida) container nursery facility. In 1985, a foliage blight ravaged the containerized longleaf pine crop, destroying thousands of seedlings. Field and laboratory analyses established a clear association between the damage and *Calonectria kyotensis* Terashita (anamorph; *Cylindrocladium floridanum* Sobers & C.P. Seymour). Isolations from necrotic pine needles consistently yielded the distinctive anamorph, and characteristic perithecia of the teleomorph were observed in profusion on symptomatic tissues in the field. Koch's postulates were not completed for this apparently unreported host-pathogen association. However, characteristic pathogenicity of *Cylindrocladium* (and *Calonectria*) spp. on a wide variety of hosts growing in closely spaced, poorly aerated, hot, humid environments (Barnard 1984) and consistent association between disease symptoms and the isolated organism leave little doubt as to the causal relationship.

Cultural practices including improved seedling container spacing, judiciously timed clippings of longleaf pine seedling needles, together with a fungicidal protection program consisting of alternating

sprays of chlorothalonil and benomyl provided adequate protection for subsequent seedling crops into the early 1990's. In 1993, the Division of Forestry relocated its containerized nursery operation into northern Florida (Levy County). This move has apparently provided a fortuitous escape from the *Calonectria/Cylindrocladium* inoculum and associated disease pressure.

Blights of Redcedar

Redcedar (*Juniperus virginiana*) was a significant seedling crop in Florida forest nurseries until very recently. Despite recent decreases in overall production, some forest nurseries still produce large numbers of bare-root seedlings. In certain nurseries, these crops have been periodically ravaged by *Phomopsis juniperovora* Hahn, necessitating consistent fungicidal applications using primarily thiophanate methyl and zinc ion + maneb.

In one nursery, serious seedling losses were once attributable to a blight caused by *Sclerotium rolfsii* Sacc. [teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough]. This host-pathogen relationship, although apparently not unknown, was unusual in a forest nursery. Roguing infected seedlings and removing substantial buffers around infection foci was successful in reducing losses.

Root Knot Nematode-Macrophomina Complex on Dogwoods

Through the 1970's and into the 1980's, flowering dogwoods (*Cornus florida*) were routinely produced as bare-root seedlings in state-owned forest nurseries in Florida. Today, dogwood seedling production is handled predominantly by the private sector. In 1981, serious damage was inflicted on one dogwood crop by a root knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood] - *M. phaseolina* complex (Barnard et al. 1982; Lehman and Barnard 1982). Losses of about 180,000 seedlings valued at more than $10,000 resulted. The disease occurred once and was restricted to a nursery area that had not been fumigated immediately prior to sowing.

Anthracnose of Acacia

At one time the Florida Division of Forestry produced a wide variety of container-grown seedling species for urban landscape beautification. Under warm, humid conditions, close seedling spacings, and frequent irrigation, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Sacc. [teleomorph: *Glomerella cingulata* (Stonem.) Spaulding and Schrenk] became a serious problem (Barnard and Schroeder 1984) on *Acacia* spp. grown at a container nusery in Highlands County (south-central Florida). Chlorothalonil provided some control, but losses were mostly reduced by lowering seedling densities and reducing irrigation.

Mycorrhizae

Artificial inoculations with selected mycorrhizal fungi are not operational procedures in Florida's forest tree nurseries. Experimental inoculations with the ectomycorrhizal symbiont *Pisolithus tinctorius* were conducted in several pine nurseries with variable results. However, rapid and abundant colonization of Florida nursery soils by *Thelephora terrestris*, *Rhizopogon nigrescens*, and sometimes *P. tinctorius* has obviated any need for artificial inoculation of pine seedlings for routine afforestation or reforestation. Although suppression of ectomycorrhizal development on pine seedlings sprayed with triadimefon for control of fusiform rust is commonplace (Marx et al. 1986), negative effects on overall seedling development and field performance have not been demonstrated.

Deficiencies in vesicular-arbuscular mycorrhizae (VAM) and associated reduced seedling growth were documented for a variety of endomycorrhizal tree species in fumigated nursery seedbeds (Barnard, unpublished). Tree species affected included *Acer rubrum* L., *Liquidambar styraciflua* L., *Cornus florida*, and *Juniperus virginiana*. Deficiencies are typically present in seedbed soils fumigated immediately prior to seed sowing. Fumigation and growing a suitable cover crop several months to a year prior to sowing have proven beneficial for alleviating VAM deficiencies.

Heat Lesions

Periodically in Florida, forest nursery seedlings suffer damage as a result of high groundline temperatures and resulting groundline heat lesions (Barnard 1990). Damage often occur on young, germinating or recently germinated seedlings, but older seedlings can also be affected. The frequency and severity of damage is clearly related to high spring and early summer temperatures. Damaged seedlings are often killed. Occurrence of damaging groundline temperatures is related not only to ambient air temperatures, but also to levels of groundline insolation, soil moisture, color of the soil surface, and irrigation patterns. For example, major seedling losses were sustained recently in one Florida forest tree nursery, apparently because of heat damage

related to a dark-colored and heat-absorbing seedbed mulch.

Nutrient Deficiencies

Crop-damaging nutrient deficiencies are rare in Florida's forest tree nurseries. Although Florida's typically sandy forest nursery soils are innately prone to deficiencies, fertilization programs are sophisticated and judiciously applied.

Always of concern to Florida's nurserymen is the maintenance of a suitable pH in nursery seedbed soils. High levels of basic cations (especially Ca) are usually found in irrigation water from wells drawing from limestone aquifers. Therefore, soil pH easily becomes alkaline. As a result, soil pH values in excess of 6.0 are not uncommon and contribute to micronutrient deficiencies, especially Fe and Mn. These pH-induced deficiencies are usually recognized quickly and treated with appropriate soil amendments, such as sulfur and micronutrient fertilizers.

One little-known and novel micronutrient deficiency which has occurred in Florida forest nurseries is boron deficiency. This infrequent, but problematic deficiency has been documented in one Florida nursery (Stone et al. 1982) and is believed to have occurred in two others (Barnard, unpublished). Affected pine seedlings (*P. taeda*; *P. elliottii*) exhibit dysfunctional and necrotic, sometimes resinimpregnated terminal buds. Outplanted seedlings initiate growth with a pronounced lack of apical dominance, resulting in a bush-like appearance. Although effects of boron deficiencies can be pronounced and serious, deficiencies in well managed nurseries are rare and usually avoided by maintaining a suitable soil pH and adhering to a systematic micronutrient fertilization program.

APPROACHES TO DISEASE MANAGMENT

Forest tree nursery disease management in Florida is a constant concern. Like so many other aspects of nursery management, disease management strategies are continuously evaluated and subject to periodic change. Changes in approaches to disease management have been directed by improved understanding of pathogen and disease biology, economics, and environmental considerations. Perhaps the single most important stimulus to the search for improved understanding of forest tree nursery disease biology and alternative strategies for disease control has been the U.S. Environmental Protection Agency's (EPA) proposal to ban the production and use of methyl bromide pursuant to the United States' Clean Air Act of 1990 (and in response to the 1991 Montreal Protocol) (Civerolo et al. 1993; Smith and Fraedrich 1993). For some, EPA's edict has invoked a measure of consternation, while for others it represents simply another challenge to overcome. In my opinion, Florida's forest tree nursery disease history provides a base for optimism with respect to the possibilities for alternative strategies. Indeed, some of the more effective strategies for disease management in Florida forest nurseries have been approaches other than "spray it, gas it, or kill it". The need for research on forest nursery diseases, insects and weeds remains great, but there is little reason to fear an "altered" future.

LITERATURE CITED

Barnard, E.L. 1979. Rhizoctonia blight of longleaf pine seedlings. Florida Department of Agriculture & Consumer Services, Division of Plant Industry. Plant Pathology Circular No. 207. 2p.

Barnard, E.L. 1984. Occurrence, impact, and fungicidal control of girdling stem cankers caused by *Cylindrocladium scoparium* on eucalyptus seedlings in a south Florida nursery. Plant Disease 68:471-483.

Barnard, E.L. 1988. Phytophthora root rot of sand pine. Florida Department of Agriculture & Consumer Services, Division of Plant Industry. Plant Pathology Circular No. 314. 4 p.

Barnard, E.L. 1990. Groundline heat lesions on tree seedlings. Florida Department of Agriculture & Consumer Services, Division of Plant Industry. Plant Pathology Circular No. 338. 2 p.

Barnard, E.L. 1994. Nursery-to-field carry over potential and post-outplant impact of *Macrophomina phaseolina* on loblolly pine on a cutover site in north central Florida. Tree Planters' Notes. 45:68-71.

Barnard, E.L., E.C. Ash and W.N. Dixon. 1995. Benomyl root dip and scalping improve early performance of longleaf pine on pest-infested agricultural croplands. Tree Planters' Notes 46:93-96.

Barnard, E.L. and G.M. Blakeslee. 1980. Pitch canker of slash pine seedlings: A new disease in forest tree nurseries. Plant Disease 64:695-696.

Barnard, E.L., G.M. Blakeslee, J.T. English, S.W. Oak and R.L. Anderson. 1985a. Pathogenic fungi associated with sand pine root disease in Florida. Plant Disease 69:196-199.

Barnard, E.L., C.E. Cordell, S.P. Gilly and A.G. Kais. 1993. Comparative performance of sand and longleaf pines on a *Phytophthora cinnamomi*-infested sandhill in west Florida. Southern Journal of Applied Forestry 17:90-95.

Barnard, E.L., W.D. Dixon, E.C. Ash, S.W. Fraedrich and C.E. Cordell. 1994a. Scalping reduces impact of soilborne pests and improves survival and growth of slash pine seedlings on converted agricultural croplands. Southern Journal of Applied Forestry. 19:49-59.

Barnard, E.L., S.W. Fraedrich and S.P. Gilly. 1994b. Interactions between seedbed mulches and seedling disease development. *In*: Landis, T.D. (tech. coord.). Proceedings of the Northeastern and Intermountain Forest and Conservation Nursery Associations. USDA Forest Service, Rocky Mountain Forest & Range Experiment Station. General Technical Report RM-243. pp. 97-104.

Barnard, E.L. and S.P. Gilly. 1986. Charcoal root rot of pines. Florida Department of Agriculture & Consumer Services, Division of Plant Industry. Plant Pathology Circular No. 290. 4 p.

Barnard, E.L., S.P. Gilly and E.C. Ash. 1994. An evaluation of dazomet and metam-sodium soil fumigants for control of *Macrophomina phaseolina* in a Florida forest nursery. Tree Planters' Notes. 45:91-94.

Barnard, E.L. and R.A. Schroeder. 1984. Anthracnose of acacia in Florida: occurrence and fungicidal control. Proceedings of the Florida State Horticultural Society. 97:244-247.

Barnard, E.L., R.A. Schroeder and P.S. Lehman. 1982. Occurrence of *Meloidogyne incognita* and *Macrophomina phaseolina* on dogwood seedlings in a Florida forest tree nursery. Southwide Forest Disease Workshop. VPI & SU, Blacksburg, VA. June 8-10, 1982. 1 p. (Abstr.)

Barnard, E.L., R.S. Webb, S.P. Gilly and W.D. Lante. 1985b. *Phytophthora cinnamomi* infection in sand pine seedlings in Florida nurseries and effects on outplant survival. *In*: South, D.B. (ed.). Proceedings of the International Symposium on Nursery Management Practices for the Southern Pines. Alabama Agriculture Experiment Station, Auburn University and International Union of Forest Research Organizations. pp. 486-495.

Blakeslee, G.M., L.D. Dwinell and R.L. Anderson. 1980. Pitch canker of southern pines: Identification and management considerations. USDA Forest Service, State and Private Forestry, Southeastern Area, Forest Report SA-FR11. 15 p.

Carey, W.A. and W.D. Kelley. 1993. Seedling production trends and fusiform rust control practices at southern nurseries, 1981 - 1991. Southern Journal of Applied Forestry 17:207-211.

Civerolo, E.L., S.K. Narang, R. Ross, K.W. Vick and L. Greczy (eds.). 1993. Alternatives to methyl bromide: Assessment of research needs and priorities. Proceedings from the USDA Workshop on Alternatives to Methyl Bromide. Arlington, VA. June 29-July 1, 1993. 85 p.

Davis, W.C. 1941. Damping-off of longleaf pine. Phytopathology 52:210-219.

Dhingra, O.D and J.B. Sinclair. 1978. Biology and pathogenicity of *Macrophomina phaseolina*. Imprensa Universitaria. Universidade Federal de Vicosa. Minas Gerais, Brazil. 166 p.

Dwinell, L.D., J.B. Barrows-Broaddus and E.G. Kuhlman. 1985. Pitch canker: a disease complex of southern pines. Plant Disease 69:270-276.

English, J.T., R.C. Ploetz and E.L. Barnard. 1986. Seedling blight of longleaf pine caused by a binucleate *Rhizoctonia solani*-like fungus. Plant Disease 70:148-150.

Gilly, S.P., E.L. Barnard and R.A. Schroedeer. 1985. Field trials for control of rhizoctonia blight on longleaf pine seedlings: Effects of seedbed planting densities, fungicides and mulches. *In*: South, D.B. (ed.). Proceedings of the International Symposium on Nursery Management Practices for the Southern Pines. Alabama Agriculture Experiment Station, Auburn University and International Union of Forest Research Organizations. pp. 486-495.

Hodges, C.S. 1962. Black root rot of pine seedlings. Phytopathology 52:210-219.

Kelley, W.D. 1985. Recommended Bayleton treatments for control of fusiform rust in forest tree nurseries. Auburn University Southern Forest Nursery Management Cooperative Research Note No. 21. 2p.

Kelley, W.D. and G.B. Runion. 1991. Control of fusiform rust on loblolly and slash pine seedlings in forest nurseries in the southeastern United States. *In*: Hiratsuka Y., J.K. Samil, P.V. Blenis, P.E. Crane and B.L. Laishley (eds.). Rusts of Pine. Proceedings of the Third IUFRO Rusts of Pine Working Party, Forestry Canada, Northwest Region Information Report NOR-X-317. pp. 338-340.

Lehman, P.S. and E.L. Barnard. 1982. A root-knot nematode pathogenic to flowering dogwood, *Cornus florida*. Florida Department of Agriculture & Consumer Services, Division of Plant Industry. Nematology Circular No. 84. 2 p.

Marx, D.H., C.E. Cordell and R.C. France. 1986. Effects of triadimefon on growth and ectomycorrhizal development of loblolly and slash pines in nurseries. Phytopathology 76:824-831.

McDonald, D. and V.K. Mehan. 1984. Charcoal rot. *In*: Porter, D.M., D.H. Smith and R. Rodriquez-Kabana (eds.). Compendium of peanut diseases. American Phytopathological Society. St. Paul, MN. 75 pp.

Powers, H.R., Jr. 1984. Control of fusiform rust of southern pines in the U.S.A. European Journal Forest Pathology. 14:426-431.

Reuveni, R. and Z. Madar. 1985. The role of *Macrophomina phaseolina* in mortality of pine seedlings in forest nurseries. Phytopathologische Zietschrift 112:161-164.

Ross, E. W. and D. H. Marx. 1972. Susceptibility of sand pines to *Phytophthora cinnamomi*. Phytopathology 62:1197-1200.

Rowan, S.J. 1971. Soil fertilization, fumigation, and temperature affect severity of black root rot of slash pine. Phytopathology 61:184-187.

Rowan, S.J. 1982a. Influence of method and rate of application of Bayleton on fusiform rust on slash pine seedlings. Tree Planters' Notes 33(1):15-17.

Rowan, S.J. 1982b. Tip dieback in southern pine nurseries. Plant Disease 66:258-257.

Seymour, C.P. 1969. Charcoal root rot of nursery-grown pines in Florida. Phytopathology 59:89-92.

Seymour, C.P. and C.E. Cordell. 1979. Control of charcoal root rot with methyl bromide in forest nurseries. Southern Journal of Applied Forestry 3:104-108.

Smith, R.S. Jr. and S.W. Fraedrich. 1993. Back to the future - pest management without methyl bromide. Tree Planters' Notes 44:87-90.

Snow, G.A., S.J. Rowan, J.P. Jones, W.D. Kelley and J.G. Mexal. 1979. Using Bayleton (Triadimefon) to control fusiform rust in pine tree nurseries. USDA Forest Service, Southern Forest Experiment Station, Research Note SO-253. 5 p.

Stone, E.L., C.A. Hollis and E.L. Barnard. 1981. Boron deficiency in a southern pine nursery. Southern Journal of Applied Forestry 6:108-112.

Watkins, G.M. 1981. Charcoal rot. *In*: Watkins, G.M. (ed). Compendium of Cotton Diseases. American Phytopathological Society, St. Paul, MN. p. 50.

Wylie, T.D. 1989. Charcoal rot. *In*: Sinclair, J.B. and P.A. Backman (eds.). Compendium of Soybean Diseases. Third Edition. American Phytopatholical Society., St. Paul, MN. pp. 30-33.

THE PRESENCE OF MYCOSPHAERELLA DEARNESSII (LECANOSTICTA ACICOLA) AS A PATHOGEN IN SPANISH FOREST NURSERIES[1]

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ABSTRACT

Detection of *Mycosphaerella dearnessii* (= *Scirrhia acicola*; anamorph: *Lecanosticta acicola*) in France in 1993 was of great concern to forest nurseries in Europe because of the potential danger to native pine and nursery crops. This pathogen was observed in northern Spain in 1942 and 1965, but no noticeable damage was observed in Spanish nurseries until recently. This pathogen was recently observed in Spainish nurseries on three pine species: *Pinus merkusii*, *P. roxburguii* and *P. densiflora*.

INTRODUCTION

Concern with possible occurrences of *Mycosphaerella dearnessii* M. E. Barr. (= *Scirrhia acicola* (Dearness) Siggers) in *Pinus radiata* D. Don stands in Spain have occurred since February, 1993 when the Department de la Sante de Forets detected the disease in *Pinus attenuata* Lemm. x *radiata* stands located in the French Landas area. Morelet (1993) and Chandelier et al. (1994) described existence of this pathogen in its asexual form (*Lecanosticta acicola* (Thum.) H. Sydow) as the causal agent of "brown-spot needle blight disease". The pathogen was initially reported in Spain by Martinez (1942). He reported occurrence of *Septoria acicola* (= *Lecanosticta acicola*) within *P. radiata* stands in the provence of Vizcaya near the French border. In 1965, Martinez and Torres described this same pathogen under the name *Lecanosticta acicola* in Spain. They cited low levels of damage on *P. halepensis* P. Mill., *P. pinaster* Ait., *P. sylvestris* L. and *P. radiata*.

I have been working with *Dothistroma pini* Hulbary since 1975 (Fernandez de Ana-Magan 1975, 1979). This pathogen causes "red-band disease" in *P. radiata*. It produces needle symptoms and damage similar to those produced by *M. dearnessii*. Both fungi also produce similar immature conidia and ascomata. To add to the confusion, J. Torres (1975, unpublished) described *Lecanosticta acicola* as the same fungus as *D. pini*. Because of these similarities and the possibility of confusing the two fungi, I undertook more detailed studies of *M. dearnessii* to determine extent of its occurrence in pine stands as well as in Spanish nurseries.

SURVEY RESULTS

Extensive samples from pine stands in northern portions of the Iberian Peninsula were evaluated. *M. dearnessii* was not detected on these samples in either its sexual or asexual form.

In November 1995, samples of damaged *P. merkussii* Junghuhn & Vriese ex Vriese, *P. roxburghii* Sarg. and *P. densiflora* Sieb. et Zucc. seedlings of Asiatic origin were studied. Seed obtained from Canada was sown in 1993 and 1994. These seedlings were produced at the Department of Genetic Improvement nursery and were destined to form a collection of pines for several "pinetums" to be located in different parts of Spain as directed by the organization Patrimonio Nacional.

Severe needle injury was observed causing partial to complete defoliation during the second year of seedling growth. Defoliation was probably responsible for seedling mortality. *Lecanosticta acicola* was identified on the damaged needles which had very similar symptomatology as red-band needle blight caused by *D. pini*. This diagnosis was verified by comparing isolates from diseased seedlings with those from *P. attenuata* x *radiata* needles supplied by P. Chandelier (Service Regional de Protection de Vegetaux, Nancy, France). To our knowledge, all infected nursery plants in Spain were destroyed.

Disease symptomotology varied among the different pine hosts. Initially, small spots of a grayish-green color appears on needles. These spots become straw-yellow in color and then light brown with chestnut-brown margins. Spots coalisce and needles become necrotic. Infected needles have green bases (near facicle sheaths), mottled middle por-

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tions and necrotic apical portions. *Pinus merkusii* was very susceptible with all seedlings killed by the pathogen. Necrotic seedlings retained their needles (which were brown and dry); fruiting bodies of the fungus were prominent and appeared as black dots. *Pinus densiflora* was less susceptible, with only about half the seedlings killed by the pathogen. Second-year seedlings were killed after having lost their needles during the first winter. Surviving seedlings were often of decreased vigor as evidenced by smaller needles following reflushing. *Pinus roxburghii* was the most resistance species. At the end of the first growing season, infected seedlings appeared somewhat chlorotic. Some seedling mortality occurred during the second growing season.

Seedling decline caused by *M. dearnessii* may be similar to that caused by other nursery pathogens such as *Phoma*, *Naemacyclus*, and *Lophodermium* spp. These secondary pathogens may often infect seedling needles, thus masking disease caused by *M. dearnessii*. The diagnostic problem is also compounded by the fact that *D. pini* may also colonize seedlings infected by *M. dearnessii*.

Fruiting bodies of *L. acicola* produced few conidia; these spores were usually not mature and were often of differing morphology (length, width, color, and shape). Production of immature spores may have been enhanced by applications of fungicides (benomyl and Bordeaux mixture) that occurred throughout the second growing season.

CONCLUSIONS

Discovery of *M. dearnessii* in European nurseries is cause for concern because of possible spread of the pathogen to native forest stands, such as those containing *P. sylvestris*. This pine species is highly susceptible to the fungus in North American Christmas tree plantations. This pathogen has been the target of eradication programs in France, Germany and Switzerland, where infected trees are removed. It is also possible that the pathogen occurs in portions of Austria and the former Yugoslavia, although confirmation is required. Disease caused by *M. dearnessii* is also common around the Gulf of Mexico, extending north to Canada and south to Colombia. The pathogen was also recently introduced into moist areas of China. Pine species that are susceptible to *M. dearnessii*, besides those reported in Spanish nurseries, include *P. sylvestris*, *P. taeda* L., *P. banksiana* Lamb., *P. contorta* Dougl., and *P. radiata*.

From this recent work, it is concluded that previous reports of *L. acicola* in Spain are probably incorrect. It is suspected that the 1942 report (Martinez) was likely *D. pini* or another closely-related fungus. The criterion of condial length is not a good diagnositc characteristic to differentiate between *L. acicola* and *D. pini*. Likewise, conidial color and shape are also not consistent characteristics useful for separating these two taxa. Conidial width is a better diagnosistic tool as is color of the hyphae colonizing needles. For example, *L. acicola* produces hyphae that are consistently warty and usually brown in color compared to the smooth, hyaline hyphae of *D. pini*.

Because of these diagnostic difficulties and current quarantines enforced by the European Union, it is important to expand surveys of susceptible pine plantations in Spain, especially those derived from seed obtained from locations where *M. dearnessii* is known to occur.

LITERATURE CITED

Chandelier, P., C. Lafaurie and F. Maugard. 1994. Decouverte en France deMycosphaerella dearnessii sur Pinus attenuata x radiata. C.R. Academy Agriculture Fr. No. 1. pp. 103-108.

Fernandez de Ana-Magan, F.J. 1975. Le enfermedad de la banda roja en el *P. radiata*. Comunicaciones INIA. Serie Proteccion Vegetal 3. 16p.

Fernandez de Ana-Magan, F.J. 1979. Estudio de los danos producidos por el *Dothistroma pini* Hulbary en masas de *Pinus radiata* D. Don en Galicia. Comunicaciones INIA, Serie Proteccion Vegetal 8. 28p.

Levy, A. and C. Lafaurie. 1994. Decouvrte de *Scirrhia acicola*. Un nouveau pathogene foliaire des *Pinus attenuata* x *radiata* en Aquitaine. Phytoma - La Defense des vegetaux. No. 463. pp. 33-35.

Martinez, J.B. 1942. Las microsis del Pinus insignis en Guipuzcoa. Instituto Forestal de Investigaciones y Experiencias, Madris. No. 23. 45 p.

Martinez, J.B. and J.J. Torres. 1965. Enfermedades de las coniferes espanolas. Instituto Forestal de Investigaciones y Experiencias, Madrid. No. 88. 68p.

Morelet, M. 1993. Note preliminaire sur quatre ascorycetes pathogenes. Extrait des Annales de la S.S.N.A.T.V., t. 45, 3ϵ trimestre. pp. 217-220.

Morelet, M. 1993. Note preliminaire sur quatre ascorycetes pathogenes. Extrait des Annales de la S.S.N.A.T.V., t. 45, 3ϵ trimestre. pp. 217-220.

FUSARIUM DISEASES

OF FOREST NURSERIES

PATHOGENICITY OF FUSARIUM PROLIFERATUM IN CONTAINER-GROWN DOUGLAS-FIR SEEDLINGS[1]

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ABSTRACT

Fusarium proliferatum (teleomorph: *Gibberella fujikuroi*) is one of the most commonly-isolated fungi from roots of both diseased and healthy container-grown conifer seedlings within nurseries in the Pacific Northwest of the United States. This fungus colonizes root cortical tissues and is particularly common on seedlings several months old. From evaluations at container nurseries, we found that most isolates of *F. proliferatum* were very similar morphologically. Much less pathogenic diversity on conifer seedlings occurred within this species than was often found with other fusaria, particularly *F. oxysporum*. Most tested isolates were very aggressive on conifer seedlings, causing damping-off and root disease. Genetic diversity among conifer seedling isolates needs evaluation. Disease-associated losses are variable among nurseries and maintaining high seedling vigor is important for limiting damage.

INTRODUCTION

Recently, production of container-grown conifer seedlings for reforestation has greatly increased in the Pacific Northwest of the United States. Conifer seedlings can be produced quickly (6-8 months) and usually perform when outplanted as well as bareroot seedlings which may take 2-3 years to produce. Unfortunately, conditions ideal for growing container seedlings are also ideal for infection and damage by several disease-causing fungi (James 1984b), particularly foliage pathogens like *Botrytis cinerea* Pers. ex Fr. (James 1984a) and root diseases caused by *Fusarium* spp. (James et al. 1987).

Several years ago we began investigating root diseases of container-grown seedlings because losses were often extensive and growers needed tools for reducing these losses. Initial efforts involved isolating and identifying root-colonizing fungi associated with diseased seedlings at various stages throughout the greenhouse growth cycle. Several different types of fungi were isolated, but the most common were in the anamorphic genus *Fusarium* (James et al. 1987). Although several different *Fusarium* spp. were encountered at different nurseries (James et al. 1989a), one species was usually represented at high numbers in all sampled nurseries. This species produced common *Fusarium*-like macroconidia in sporodochia, chains of microconida borne either in mono- or polyphialides, lacked typical chlamydospores and often produced various shades of violet pigment in pure culture. Superficially, cultures of this species resembled *F. oxysporum* Schlecht., a species commonly encountered causing root disease of conifer seedlings, especially bareroot stock (James et al. 1989a, 1991). Based on descriptions of Nelson et al. (1983), we classified this fungus in the section *Liseola* and designated it in the anamorphic taxon *F. proliferatum* (Matsushima) Nirenberg.

Over several years, at different container nurseries, we collected many isolates of *F. proliferatum* that appeared quite similar morphologically when grown in pure culture on potato dextrose agar (PDA). The only morphological variability we encountered was level of violet pigmentation and extent of polyphialide production. This lack of variability contrasts with other fusaria we have commonly isolated, particularly those classified as *F. oxysporum* (James et al. 1989a), which varies widefly in extent of aerial mycelium, pigmentation, sporodochial production, and microconidial morphology.

We found that *F. proliferatum* was more commonly isolated later in the crop cycle when seedlings were several months old, rather than on very young seedlings (James et al. 1995). We also found that this species was infrequently isolated from conifer seed (James et al. 1995). Apparently, the species was

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introduced at high levels to seedling crops in some other way.

Since most nurseries use containers for several seedling crops, we began to look at reused containers as a potential source of root disease inoculum. Most nurseries superficially washed containers with high-pressure water (sometimes steam) to remove residual organic debris (growing media, seedling roots) remaining after seedling extraction. This type of "cleaning" was unsatisfactory because relatively high levels of fungal inoculum remained, particularly *F. proliferatum*, on the inner walls of both styroblock and plastic cell containers (James et al. 1988). Although chlamydospore production is lacking in *F. proliferatum* (Nelson et al. 1983), the fungus is capable of surviving within containers for several months, probably colonizing organic debris remaining after "cleaning" (James et al. 1995). Immersing containers in hot water renders most potential disease inoculum, including *F. proliferatum*, nonviable (James et al. 1988; James and Woollen 1989).

Despite treating containers with hot water, we still encountered *F. proliferatum* on roots of container seedlings by the end of the growth cycle, even those without typical disease symptoms (James et al. 1995). *F. proliferatum* may be disseminated throughout greenhouses on bodies of adult fungus gnats (James 1994), but we suspect that passive dissemination of this fungus is most common. In particular, the fungus produces long chains of microconidia that dislodge upon drying to be carried by air currents. It is also possible that some inoculum may be brought into greenhouses from outside sources. Because *F. proliferatum* commonly infects apparently healthy seedlings, we hypothesized that some isolates might be more pathogenic than others, similar to the situation that occurs with *F. oxysporum* (Gordon and Okamoto 1992; James et al. 1989b). The remainder of this paper describes our efforts to test our hypothesis and discusses our findings in the context of what we know about *F. proliferatum* epidemiology.

MATERIALS AND METHODS

One laboratory and three greenhouse experiments were conducted to evaluate pathogenicity of *F. proliferatum*. In all experiments, Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seeds (seedlot FN, University of Idaho, Moscow, Idaho) were used. All seeds were soaked in a two-part bleach and three-part water solution for 10 min (Wenny and Dumroese 1987), rinsed 48 h in running tap water, and stratified 21 days at 2-3°C.

Inoculum for the experiments was prepared using the techniques of Miles and Wilcoxon (1984). Basically, this techniques uses perlite, an inert, inorganic, siliceous rock of volanic origin, commonly used in potting mixtures, as the matrix for fungal growth. To provide nutrients for fungal growth, cornmeal (150g) was moistened with warm 1% potato dextrose agar (PDA) (300ml) and added to perlite (75g). Perlite/cornmeal/PDA mixtures were inoculated with 10ml spores suspensions of selected *Fusarium* isolates (prepared by adding sterile, distilled water to 14-day-old cultures) and incubated in the dark at about 24°C for at least 21 days, after which the fungus had thoroughly colonized the perlite/cornmeal substrate. After incubation, inoculum was dried in open petri plates within a cabinet. After 5-7 days, inoculum was dry and uncontaminated because the food base was completely colonized by the inoculated fungus. Inoculum was stored in plastic vials under refrigeration until needed, remaining viable for at least 2 years.

For all greenhouse experiments, Douglas-fir seedlings were grown in a 1:1 peat:vermiculite growing medium (W. R. Grace & Co., Portland, Oregon), commonly used to grow conifer seedlings, using a standard growing regime (Wenny and Dumroese 1992).

Laboratory experiment:

Twenty-four isolates of *F. proliferatum* obtained from roots of diseased or non-diseased container-grown seedlings were tested for pathogenicity using a previously described lab technique (James 1996). Basically, glass vials (23ml) were filled to about two-thirds capacity with a dried coconut:vermiculite medium (Grace/Sierra Horticultural Productes, Milpitas, CA) and autoclaved 60 min at 121°C. For each isolate, inoculum was ground to a fine powder with mortar and pestle and 0.05g was added to each of 24 vials of autoclaved medium, resulting in an aproximately 1:50 (w/w) mixture of inoculum to medium. Inoculum was distributed throughout the medium by shaking. For each pathogenicity test sequence, one set of 24 control vials was made using non-inoculated perlite instead of inoculum.

Douglas-fir seeds, after stratification, were placed on moistened sterile filter paper (Whatman No. 3, Whatman International Ltd., Maidstone, England) within sterile petrie dishes and incubated under 12 hr. diurnal fluorescent light at about 24°C. Germinants, selected when their primary root was at least 3mm long, were carefully placed with their primary root pointing downward, one per vial. Four ml sterile

water were added to each vial just before germinant placement. We incubated vials with germinants under cool, fluorescent, diurnal light (12 h photoperiod) at 22-24°C. Germinants within inoculation vials were examined daily for disease symptoms (damping-off and hypocotyl rot). Two ml sterile water were added to germinants surviving 7 days. Tests concluded at 14 days, and all surviving germinants were removed and examined for disease symptoms. Reisolations were made from all inoculated germinants on a selective agar medium (Komada 1975) to determine if infection by inoculated isolates occurred.

A numerical rating system was developed to compare levels of *Fusarium* virulence (James 1996). The rating system awarded points based on duration of germinant survival within inoculated vials, occurrence and type of disease, reisolation of inoculated fungal isolate, and primary root growth within the vial. Germinants received one point for each day of survival from day 3-14 (12 points maximum), another 1, 2 or 4 points depending on type of disease apparent (maximum points if non-diseased), an additional 1, 2 or 4 points based on fungal reisolation (maximum points if no organism reisolated), and 3 points more if the root grew to the bottom of the vial. The range of possible points was 3-23, with higher point values reflecting less aggression by the tested isolate against the germinant. To convert points to a score in which higher numbers represent greater virulence, we devised a reciprocal rating. Ratings ranged from 0 to 100; 0 indicating germinants were uninfected within 14 days and 100 indicating all germinants killed within 3 days by the inoculated isolate.

Greenhouse experiment:

We conducted three greenhouse pathogenicity experiments on seedlings of various ages: germinants, 2 months old, and 5 months old. Inoculum was prepared for two isolates each of *F. proliferatum* and *F. oxysporum*. The first *F. proliferatum* isolate (8629G) was obtained from non-diseased Douglas-fir seed (seedlot FN), while the second isolate (8656M) was from roots of a non-diseased Douglas-fir seedling (seedlot FN). Both *F. oxysporum* isolates were obtained from roots of diseased Douglas-fir seedlings growing at the USDA Forest Service nursery, Coeur d'Alene, Idaho (isolate 8649S from seedlot 6070 and isolate 8675B from seedlot 2741). Results of some of these tests using other *F. oxysporum*, *F. acuminatum* Ell. & Ev., *F. avenaceum* (Fr.) Sacc., and *F. sambucinum* Fuckel isolates were previously reported (James et al. 1989b).

Germinant experiment:

Metal trays (38cm x 23cm x 5cm) were filled with peat:vermiculite, autoclaved 60 minutes at 121°C, and allowed to cool. Inoculum was mixed thoroughly into the autoclaved medium at a 1:25 or 1:50 (w/w) basis. Controls consisted of autoclaved growing medium amended with uninoculated perlite at the same two treatment concentrations. The inoculum-growing medium mixture was moistened with sterile distilled water until the mixture was at field capacity.

Seeds were treated as described above, but after the bleach treatment and before the 48 hr. running water rinse, seeds were also soaked 3 minutes in a 70% ethanol solution. After stratification, 200 seeds were sown into each tray and covered with 5mm layer of silica grit. Trays were then placed inside the greenhouse.

Germination and seedling emergence were monitored in each tray; post-emergence damping-off was determined by examining trays for seedlings with stem lesions. Damped-off seedlings were carefully removed and used to isolate inoculated fungal isolates to confirm pathogenicity. All isolations were made on a selective medium for *Fusarium* spp. (Komada 1975).

Germination was considered complete 30 days after sowing and ten non-diseased seedlings, if that many were still available, were then collected from each tray for root isolations. Also, ten ungerminated seed were carefully removed from each tray for determination of infection by inoculated fungal isolates. Isolations were made onto the selective medium and emerging fungi were compared with inoculated isolates to confirm root and seed infection.

Two-month-old seedling experiment:

Seedlings were grown in 66ml Spencer-LaMeire Root Trainers for 7 weeks and then carefully removed from their containers. Roots were gently and thorougly washed to removed adhering particles of growing medium and repotted in Ray Leach pine cells (66ml) with a mixture of peat:vermiculite amended with inoculum at a concentration of 1:50 (w/w). Twenty-five seedlings were inoculated per isolate; controls consisted of repotting seedlings in an uninoculated perlite-growing medium mixture. As seedlings died, we removed them for root isolations to determine if infection was by inoculated isolates. All remaining seedlings were rated for se-

verity 84 days after inoculating using a five-point system describing extent of foliar chlorosis and necrosis (James et al. 1989b). The higher the rating, the more severe the disease symptoms. After rating, isolations were made from all seedlings' roots to determine infection by inoculated isolates.

Five-month-old seedling experiment:

Seedlings were grown in Ray Leach pine cells for 5 months and then carefully removed from their containers. Roots were gently and thoroughly washed to remove adhering particles of growing medium and repotted into Ray Leach super cells (144ml) with a mixture of peat:vermiculite amended with inoculum at a concentration of 1:50 (w/w). Ten seedlings were inoculated per test isolate; controls consisted of repotting seedlings in an uninoculated perlite-growing medium mixture. As seedling died, we removed them for root isolations to determine if infection was by inoculated isolates. Seedlings were rated using the five-point disease severity system, and roots isolated as described above 68 days after inoculation.

RESULTS AND DISCUSSION

Laboratory experiment:

All tested *F. proliferatum* isolates had virulence ratings of high or moderate on young Douglas-fir germinants (Table 1). For some isolates, three different tests were conducted and, virulence was usually quite similar in all tests. No apparent effects of host source or disease condition of host was noticed on relative virulence of these isolates. In general, most *F. proliferatum* isolates evaluated were consistently high in virulence with little variation among all the tested isolates. This contrasts similar tests of soilborne and conifer seedling root tissue isolates of *F. oxysporum*, which consistently show a very wide range of virulence from non-pathogenic to highly virulent (James, unpublished).

Greenhouse experiments:

Comparisons of selected *F. proliferatum* and *F. oxysporum* isolates indicated that the former species was more pathogenic than the latter in tests evaluating Douglas-fir seed germination and post-emergence damping-off (Table 2), and disease severity on young (Table 3) and older (Table 4) seedlings. *F. proliferatum* test isolates resulted in reduced germination and higher post-emergence damping-off than typical *F. oxysporum* isolates (Table 2). Likewise, the same *F. proliferatum* isolates resulted in greater mortality of young Douglas-fir seedlings and overall higher disease ratings (Table 3), as well as higher disease ratings of 5-month-old seedlings (Table 4). Although only a few isolates were evaluated in these latter tests, the data suggests that *F. prolferatum* may be more virulent in controlled pathogenicity tests than typical *F. oxysporum* isolates.

From these experiments, we conclude that anamorphic isolates of *F. proliferatum* are important, potentially highly virulent pathogens of container-grown conifer seedlings. Douglas-fir is especially susceptible to damage, although many other conifer species are also affected. Reducing damage caused by *F. proliferatum* may be difficult because by the time disease symptoms occur on seedlings, most root systems are thoroughly colonized with the pathogen. Fungicide applications when disease symptoms become noticeable are usually ineffective (Dumroese et al. 1990), probably because of the extent of root colonization and the fact that much of the active ingredient is incapable of reaching root systems in enough concentration to eliminate the pathogen. Likewise, biological control agents have thus far proved ineffective (Dumroese et al. 1996). We recommend prevention as the most reliable means of reducing damage. Prevention requires an aggressive program of sanitation, including sterilizing containers which may be reused for additional seedling crops (James et al. 1988), cleaning greenhouse benches, floors and walls with a surface sterilization agent such as bleach, and monitoring disease carefully, including removal of diseased seedlings to reduce potential secondary pathogen spread (James et al. 1991). Such techniques have worked well in some nurseries to reduce disease severity.

More work is needed in understanding the epidemiology of *F. proliferatum*- associated diseases. We need to know more about inoculum sources, factors affecting seedling root infection, and potential ameliorating effects of other microorganisms that might induce antagonism. As chemical pesticide use continues to decrease because of environmental and worker safety concerns, growers will have to rely on cultural disease controls as well as implementing effective biological controls as part of integrated pest management systems to reduce impacts of root diseases caused by *F. proliferatum*.

Table 1. Virulence of selected *Fusarium proliferatum* isolates on young Douglas-fir germinants in the laboratory.

	Source[1]			Percent			
Isolate	Host	Conditon	Test No.	Percent Diseased	Avg. [2] Survival	Avg. Score	Virulence[3] Rating
9036B	DF	Diseased	1 2 3	100 100 100	5.4 4.8 3.1	87 90 99	High High High
9061C	DF	Healthy	1 2	100 100	6.6 3.0	81 98	High High
9104C	DF	Healthy	1 2	100 100	3.5 3.0	96 98	High High
9108B	WL	Diseased	1 2	93 79	9.8 8.3	63 70	Mod. Mod.
9112F	BS	Diseased	1	100	5.8	84	High
9125D	WL	Diseased	1 2	100 100	3.3 3.9	98 94	High High
9131A	LP	Diseased	1 2 3	93 92 100	9.5 8.2 5.2	65 69 85	Mod. Mod. High
9131B	LP	Diseased	1 2 3	80 63 87	9.2 10.5 7.9	61 52 72	Mod. Low Mod.
9139H	WB	Diseased	1	100	4.4	92	High
9201E	GN	-	1 2 3	100 100 100	5.6 3.3 3.4	86 98 98	High High High
9202C	CT	-	1 2	100 92	3.8 5.6	94 85	High High
9202D	CT	-	1 2	100 100	3.8 3.7	95 96	High High
9202F	CT	-	1 2 3	100 100 100	3.9 3.1 3.1	95 99 99	High High High
9202H	CT	-	1 2	100 100	4.5 3.0	93 99	High High
9202I	CT	-	1	100	4.2	93	High
9202J	CT	-	1 2	100 92	5.6 5.2	85 87	High High
9202-O	CT	-	1	100	4.0	94	High
9202T	CT	-	1 2 3	100 100 100	4.0 3.2 3.3	93 99 99	High High High

Table 1. continued

	Source[1]			Percent			
Isolate	Host	Conditon	Test No.	Percent Diseased	Avg. [2] Survival	Avg. Score	Virulence[3] Rating
9223F	AS	Diseased	1	100	3.0	100	High
9224A	DF	Diseased	1	100	3.1	98	High
9225P	WB	Diseased	1	100	4.0	92	High
9249P	DF	Healthy	1	100	3.8	96	High
9306C	WP	Diseased	1	100	3.2	99	High

[1] All hosts were container-grown seedlings except GN = fungus gnats trapped in greenhouses and CT = styroblock containers used to grow seedlings. DF = Douglas-fir, WL = western larch (*Larix occidentalis* Nutt.), BS = Colorado blue spruce (*Picea yungens* Englem.), LP = lodgepole pine (*Pinus contorta* Dougl.), WB = whitebark pine *Pinus albicaulis* Englem.), AS = aspen (*Populus tremuloides* Michx.), WP = western white pine (*Pinus monticola* Dougl.).

[2] Average number of days germinants survived; all tests ran for 14 days.

[3] Virulence scores ranged from 0 to 100; the higher the number, the more virulent the isolate. See James 1996 for complete descriptions of the numerical point system and derivation of virulence scores.

Table 2. Effects of selected *Fusarium proliferatum* and *F. oxysporum* isolates on germination of Douglas-fir seed and post-emergence damping-off of young seedlings in the greenhouse.

Species	Isolate	Inoculum Concentration	Percent Germination	Percent Damping-off
F. proliferatum	8629G	1:25 1:50	15 15	97 97
	8656M	1:25 1:50	6 17	100 94
F. oxysporum	8649S	1:25 1:50	25 43	33 32
	8675B	1:25 1:50	29 24	35 46
Controls		1:25 1:50	72 69	1 1

Table 3. Effects of selected *Fusarium proliferatum* and *F. oxysporum* isolates on disease severity in young, inoculated Douglas-fir seedlings in the greenhouse.

Species	Percent Isolate	Mortality	Average Disease Rating*	Percent Root Colonization**
F. proliferatum	8629G 8656M	92 96	4.7 4.9	100 100
F. oxysporum	8649S 8675B	24 32	2.8 3.0	100 100
Controls		0	1.6	0

* Rating system from 1-5; higher numbers mean more severe disease symptoms.
** Seedlings not killed by either *Fusarium* spp. within 84 days of inoculation. Root colonization is the percent of sampled root system colonized by inoculated isolates.

Table 4. Effects of selected *Fusarium proliferatum* and *F. oxysporum* isolates on disease severity in older inoculated Douglas-fir seedlings in the greenhouse.

Species	Isolate	Average Disease Rating*	Percent Root Colonization**
F. proliferatum –	8629G 8656M	3.0 3.5	85 99
F. oxysporum	8649S 8675B	2.0 1.6	77 87
Controls		2.0	25

* Rating system from 1-5; higher numbers mean more severe disease symptoms.
** Seedlings not killed by either *Fusarium proliferatum* or *F. oxysporum* within 68 days of inoculation. Root colonization is the percent of sampled root system colonized by inoculated isolates (for controls: percent of sampled root system colonized by any *Fusarium* spp.).

LITERATURE CITED

Dumroese, R.K., R.L. James and D.L. Wenny. 1990. Trial of a granular etridiazole and thiophanate-methyl mixture to control *Fusarium* root disease of container-grown Douglas-fir seedlings. New Forests 4:231-236.

Dumroese, R.K., R.L. James and D.L. Wenny. 1996. *Gliocladium virens* in an alginate prill ineffective as a biological control of Fusarium root disease in container-grown Douglas-fir. New Forests 12:113-114.

Gordon, T.R. and D. Okamoto. 1992. Population structure and the relationship between pathogenic and nonpathogenic strains of *Fusarium oxysporum*. Phytopathology 82:73-77.

James, R.L. 1984a. Biology and management of Botrytis blight. *In*: Murphy, P.M. (compiler). The Challenge of Producing Native Plants for the Intermountain Area. Proceedings: Intermountain Nurseryman's Association 1983 Conference. USDA Forest Service, General Technical Report, INT-168. pp. 39-43.

James, R.L. 1984b. Diseases of containerized conifer seedlings. *In*: Dubreuil, S.H. (compiler). Proceedings of the 31st Western International Forest Disease Work Conference, Coeur d'Alene, ID. pp. 17-23.

James, R.L. 1994. Fungi carried by adult fungus gnats (Diptera: Sciaridae) in Idaho greenhouses. USDA Forest Service, Northern Region, Forest Pest Management. Report 94-5. 10p.

James, R.L. 1996. Technique for quantifying virulence of *Fusarium* and *Cylindrocarpon* spp. on conifer germinants. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 132. 8p.

James, R.L., R.K. Dumroese, C.J. Gilligan and D.L. Wenny. 1989b. Pathogenicity of *Fusarium* isolates from Douglas-fir seed and container-grown seedlings. Idaho Forest, Wildlife and Range Experiment Station. Bulletin No. 52. 10p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1988. Occurrence and persistence of *Fusarium* within styroblock and Ray Leach containers. *In*: Landis, T.D. (tech. coord.). Proceedings: Combined Meeting of the Western Forest Nursery Associations. USDA Forest Service, General Technical Report, RM-167. pp. 145-148.

James, R.L., R.K. Dumroese and D.L. Wenny. 1989a. Occurrence, characterisitcs, and descriptions of *Fusarium* isolates from Douglas-fir seed and seedlings. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-4. 23p.

James, R. L., R.K. Dumroese and D.L. Wenny. 1991. *Fusarium* diseases of conifer seedlings. *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada. Pacific and Yukon Region. Information Report BC-X-331. pp. 181-190.

James, R.L., R.K. Dumroese and D.L. Wenny. 1995. *Fusarium proliferatum* is a common, aggressive pathogen of container-grown conifer seedlings. Phytopathology 85:1129.

James, R.L., R.K. Dumroese, D.L. Wenny, J.F. Myers and C.J. Gilligan. 1987. Epidemiology of *Fusarium* on containerized Douglas-fir seedlings. 1. Seed and seedling infection, symptom production, and disease progression. USDA Forest Service, Northern Region, Forest Pest Management. Report 87-13. 22p.

James, R.L. and R.L. Woollen. 1989. An evaluation of the efficacy of hot water-chemical treatments to clean styroblock containers - Champion Timberlands Nursery, Plains, Montana. USDA Forest Service, Northern Region, Forest Pest Management. Report 89-5. 8p.

Komada, H. 1975. Development of a selective medium for quantifying isolation of *Fusarium oxysporum* from natural soil. Review Plant Protection Research (Japan) 8:114-125.

Miles, M.R. and R.D. Wilcoxson. 1984. Production of fungal inoculum using a substrate of perlite, cornmeal, and potato dextrose agar. Plant Disease 68:310.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

Wenny, D.L. and R.K. Dumroese. 1987. Germination of conifer seeds surface sterilized with bleach. Tree Planters' Notes 38(3):18-21.

Wenny, D.L. and R.K. Dumroese. 1992. A growing regime for container-grown Douglas-fir seedlings. Idaho Forest, Wildlife & Range Experiment Station. Bulletin No. 19. 8p.

A SHORT REVIEW OF *FUSARIUM* SECTION *LISEOLA*: IMPLICATIONS FOR CONIFER SEEDLING PRODUCTION[1]

R. L. James[2]

ABSTRACT

Species of *Fusarium* in the section *Liseola* have the same teleomorph (*Gibberella fujikuroi*) and similar anamorphic characteristics, including production of microconidia in chains or false heads borne on mono- or polyphialides. None of the species normally produce chlamydospores. Using anamorphic characteristics to separate taxa is difficult, so species have been delineated based on "mating populations," i.e., production of the ascigerous stage when paired with known testers. Anamorphic species designated as *F. proliferatum* reside in mating populations C and D; all isolates tested from Pacific Northwest conifer seedlings are in mating population D. Some pathogens formerly designated as *F. moniliforme* may actually be *F. proliferatum*; ambiguity can be reconciled using mating population tests and molecular nucleic acid analyses. Based on molecular analyses, *F. oxysporum*, an important pathogen of bareroot forest nurseries, is most closely related to fungi in the section *Liseola*.

REVIEW

Fusarium proliferatum (Matsushima) Nirenberg is one of several taxa placed in the *Fusarium* section *Liseola* (Leslie 1995; Nelson et al. 1983). *Liseola* contains several closely-related anamorphic taxa, all having the teleomorph *Gibberella fujikuroi* (Correll et al. 1989; Joffe 1974; Klittich and Leslie 1992; Kuhlman 1982; Puhalla and Spieth 1985; Tudzynski et al. 1996), sometimes previously called *G. moniliforme* (Snyder and Hansen 1945). Most workers now regard it as *G. fujikuroi* (Klittich and Leslie 1988; Sidhu 1983; Windels 1991). Biological species within *G. fujikuroi* have been designated as "mating populations" based on production of the sexual (ascigenous) stage when isolates are crossed with known testers (Klittich and Leslie 1992; Leslie 1991). From such crosses, several mating populations have been designated for fungi in *G. fujikuroi* (Klittich and Leslie 1992; Leslie 1991; Xu et al. 1995). Fungi with *F. proliferatum* anamorphs are found in two mating populations: C and D (Leslie 1991; 1995; Xu et al. 1995). All evaluated isolates obtained from conifer seedlings and evaluated belong in mating population D (Leslie, personal communication).

Undoubtedly, some fungi in *Liseola* were previously misidentified based solely on anamorphic characteristics (Leslie 1991, 1995). For example, *F. moniliforme*, a species very commonly isolated from maize (Logreico and Bottalico 1988), sorghum (Jardine and Leslie 1992), corn (Headrick et al. 1990; Lim 1967; Nava Saucedo et al. 1989; Ooka and Kommedahl 1977), figs (Michailides and Morgan 1994; Subbarao and Michailides 1992), asparagus (Elmer and Ferrandino 1992; La Mondia and Elmer 1989), cassava (Msikita et al. 1996) and occasionally from conifer seedlings (Chakravarty and Unestam 1986; Hildebrand 1985; Huang and Kuhlman 1990; Unestam et al. 1987) differs from *F. proliferatum* only on the basis of formation of microconidia exclusively on monophialides (Nelson et al. 1990; Tiedt and Jooste 1988). *F. proliferatum* forms microconidia on either mono- or polyphialides (Fisher et al. 1983; Klittich and Leslie 1992; Leslie 1991; Nelson et al. 1983). Conidiophores may sometimes be difficult to find and some workers have had to reidentify *F. moniliforme* isolates as *F. proliferatum* (Abbas et al. 1988; Elmer 1990; Marasas et al. 1988; Schreuder et al. 1995; Viljoen et al. 1995; Wagih et al. 1989). Because of this ambiguity, several reports designating *F. moniliforme* as the causal agent of a specific plant disease may actually have dealt with *F. proliferatum* or some other member of *Liseola* (Elmer 1991; Logreico et al. 1995; Puhalla and Spieth 1983). Fortunately, future use of the mating population concept should reduce this taxonomic ambiguity (Elmer 1995a; Leslie 1995; Plattner et al. 1996).

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Molecular analyses have simplified isolate differentiation within *Fusarium* section *Liseola*. These analyses usually evaluate nucleic acid similarities and determine if polymorphisms occur at specific sites in either nuclear, ribosomal, or mitochondrial DNA or RNA (Ellis 1988a, 1988b, 1989; Guadet et al. 1989; Leslie and Dickman 1991; Lodolo et al. 1992; Manicom et al. 1987; Marshall and Partridge 1981a, 1981b; Peterson 1991). Depending on the technique, molecular results generally substantiate identifications based on mating populations (Klittich and Leslie 1992; Leslie 1995; Xu et al. 1995). Genetic work with this group of commercially-important fungi will undoubtedly continue to clarify phylogenetic relationships.

As indicated previously, *F. moniliforme* may infect several grain crops (Kingsland and Wernham 1962) and be particularly detrimental because some isolates produce mycotoxins (Abbas et al. 1995; Jardine and Leslie 1992; Marasas et al. 1979, 1986, 1988; Miller et al. 1993; Van Asch et al. 1992). Several mycotoxins have been identified; some may cause serious diseases to mammals (Klittich and Leslie 1989). Likewise, *F. proliferatum* has also been implicated as causing diseases of assorted plants including sorghum, maize, corn, asparagus, gladiolus, and taro (Elmer 1990, 1992, 1995b; Magie 1980; Ocumb and Kommedahl 1994; Schreuder et al. 1995; Wagih et al. 1989; Wall and Cruz 1991 and some isolates produce important mycotoxins (Logreico and Bottalico 1988; Logreico et al. 1995; Marasas et al. 1986; Marin et al. 1995; Van Asch et al. 1992). *F. proliferatum* has also been reported as a biological control agent, controlling grape powdery mildew (Falk et al. 1996).

There are several references of conifer diseases associated with members of the *Fusarium* section *Liseola*. Perhaps the most common is pitch canker disease caused by *F. subglutinans* f. sp. *pini* (Correll et al. 1991; Kuhlman et al. 1978; Storer et al. 1995) which occurs in epidemic proportions in the Southeast U.S. and along the California coast. The pathogen normally causes most damage to older, natural or plantation, trees (Barrows-Broaddus 1990; Storer et al. 1995), although damage in nurseries has been recorded (Blakeslee et al. 1981; Rowan 1982; Viljoen et al. 1994). Several other conifer seedling diseases have been attributed either to *F. moniliforme* and *F. proliferatum* (Chakravarty and Unestam 1986; Fraedrich and Miller 1995; Hildebrand 1985; Huang and Kuhlman 1990; James 1991, 1992, 1993, 1995; Unestam et al. 1987; Viljoen et al. 1995).

Experience in Pacific Northwest nurseries indicates that the most common pathogen in the genus *Fusarium* causing root diseases in bareroot nurseries is *F. oxysporum* (James 1996; James et al. 1991, 1996). Experience in container nurseries indicates that *F. oxysporum* is often a major seed-borne pathogen (James 1987; James et al. 1987, 1991) and an important cause of both pre- and post-emergence damping-off (James 1993; James et al. 1987, 1991). However, as the container seedling growth cycle progresses, occurrence of *F. oxysporum* on roots of seedlings decreases, whereas infection by *F. proliferatum* often increases (James et al. 1995). By the time seedling height growth is terminated and seedlings are stressed to initiate bud set and dormancy, *F. proliferatum* commonly colonizes roots of many seedlings (James et al. 1991, 1995). During this period of seedling stress, root disease symptoms may become common on infected seedlings. However, not all infected seedlings display symptoms, even though much of their root systems may be colonized with potentially-pathogenic *F. proliferatum* (James et al. 1995). When non-diseased Douglas-fir seedlings are outplanted on forest sites, *F. proliferatum* tends to gradually decline on root systems; the fungus usually does not readily colonize new roots being formed after outplanting and gradually dies out as "plug" roots become replaced (Dumroese et al. 1993). However, when infected container seedlings are transplanted in nursery soil, especially soil fumigated with general biocides, *F. proliferatum* may become active and contribute to seedling mortality (James 1991, 1995). Introducing infected seedlings into soil that may be naturally suppressive to the pathogen (forest soil) probably results in inactivation and replacement of the pathogen by other mycoflora (Dumroese et al. 1993). However, when introduced into disease-conducive nursery soil, there may be an absence of competing mycoflora (James 1991, 1995).

Occurrence of *F. proliferatum* is rare in nursery soil and on conifer seed. *F. proliferatum* is likely introduced into greenhouses from several inoculum sources. Reused plastic and styrofoam containers are important inoculum sources (Dumroese et al. 1995; James and Woollen 1988; James et al. 1988). However, other sources such as organic debris located within greenhouses or "alternate" hosts located near greenhouses may also provide primary inoculum.

Molecular analyses indicate that isolates of *F. oxysporum* are most closely related to fungi in the section *Liseola* (Donaldson et al. 1995; Ellis 1988a,

1988b; Guadet et al. 1989; Waalwijk et al. 1996). Some workers believe that the teleomorph for *F. oxysporum*, when found, will be a species of *Gibberella* (Guadet et al. 1989). Fungi in both sections *Liseola* and *Elegans* (*F. oxysporum*) share the propensity to colonize cortical cells of conifer seedlings (James et al. 1991). Likewise, they may be present on seedlings that display disease symptoms as well as those lacking symptoms (James et al. 1987, 1991). Occurrence of a wide range of virulence in isolates of *F. oxsporum* has been well documented (Bloomberg 1976; Gordon and Okamoto 1992; James et al. 1989). However, our tests on *F. proliferatum* although limited, indicated that most isolates were capable of eliciting germinant and seedling diseases readily under experimental conditions without the variability of virulence experienced with *F. oxysporum* isolates (James et al. 1997).

LITERATURE CITED

Abbas, H.K., C.J. Mirocha, T. Kommedahl, P.M. Burnes, R.A. Meronuck and R. Gunther. 1988. Toxigenicity of *Fusarium proliferatum* and other *Fusarium* species isolated from corn ears in Minnesota. Phytopathology 78:1258-1260.

Abbas, H.K., T. Tanaka and S.O. Duke. 1995. Pathogenicity of *Alternaria alternata* and *Fusarium moniliforme* and phytotoxicity of AAL-toxin and fumonisin B1 on tomato cultivars. Journal of Phytopathology 143:329-334.

Barrows-Broaddus, J. 1990. Colonization of cones and seed of loblolly pine following inoculation with *Fusarium subglutinans*. Plant Disease 74:1002-1005.

Blakeslee, G.M., T. Miller and E.L. Barnard. 1981. Pitch canker in forest tree nurseries. USDA Forest Service, Pest Alert. Forestry Bulletin SA-FB/P 22. 1p.

Bloomberg, W.J. 1976. Distribution and pathogenicity of *Fusarium oxysporum* in a forest nursery soil. Phytopathology 66:1090-1092.

Chakravarty, P. and T. Unestam. 1986. Role of mycorrhizal fungi in protecting damping-off of *Pinus sylvestris* seedlings. *In*: Proceedings 1st European Symposium on Mycorrhizae: Physiology and Genetics. INRA, Paris. pp. 811-814.

Correll, J.C., T.R. Gordon, A.H. McCain, J.W. Fox, C.S. Koehler, D.L. Wood and M.E. Schultz. 1991. Pitch canker disease in California: pathogenicity, distribution, and canker development on Monterey pine (*Pinus radiata*). Plant Disease 75:676-682.

Correll, J.C., C.J. Klittich and J.F. Leslie. 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). Mycological Research 93:21-27.

Donaldson, G.C., L.A. Ball, P.E. Axelrood and N.L. Glass. 1995. Primer sets developed to amplify conserved genes from filamentous ascomycetes are useful in differentiating *Fusarium* species associated with conifers. Applied and Environmental Microbiology 61:1331-1340.

Dumroese, R.K., R.L. James and D.L. Wenny. 1993. *Fusarium* root infection of container-grown Douglas-fir: effect on survival and growth of outplanted seedlings and persistence of the pathogen. New Forests 7:143-149.

Dumroese, R.K., R.L. James and D.L. Wenny. 1995. Interactions between copper-coated containers and Fusarium root disease: a preliminary report. USDA Forest Service, Northern Region, Insect and Disease Management. Report 95-9. 8p.

Ellis, J.J. 1988a. Lesser known species in section *Liseola* of *Fusarium* and near relatives in the *Fusarium oxysporum* group. Mycologia 80:734-738.

Ellis, J.J. 1988b. Section *Liseola* of *Fusarium*. Mycologia 80:255-258.

Ellis, J.J. 1989. An alignment of toxigenic *Gibberella* strains having anamorphs in section *Liseola* of *Fusarium*. Mycologia 81:307-311.

Elmer, W.H. 1990. *Fusarium proliferatum* as a causal agent in Fusarium crown and root rot of asparagus. Plant Disease 74:938.

Elmer, W.H. 1991. Vegetative compatibility groups of *Fusarium proliferatum* from asparagus and comparisons of virulence, growth rates, and colonization of asparagus residues among groups. Phytopathology 81:852-857.

Elmer, W.H. 1992. Suppression of Fusarium crown and root rot of asparagus with sodium chloride. Phytopathology 82:97-104.

Elmer, W.H. 1995a. A single mating population of *Gibberella fujikuroi* (*Fusarium proliferatum*) predominates in asparagus fields in Connecticut, Massachusetts, and Michigan. Mycologia 87:68-71.

Elmer, W.H. 1995b. Association between Mn-reducing root bacteria and NaCl applications in suppression of Fusarium crown and root rot of asparagus. Phytopathology 85:1461-1467.

Elmer, W.H. and F.J. Ferrandino. 1992. Pathogenicity of *Fusarium* species (section *Liseola*) to asparagus. Mycologia 84:253-257.

Falk, S.P., R.C. Pearson, D.M. Gadoury, R.C. Seem and A. Sztejnberg. 1996. *Fusarium proliferatum* as a biocontrol agent against grape downy mildew. Phytopathology 86:1010-1017.

Fisher, N.L., W.F.O. Marasas and T.A. Toussoun. 1983. Taxonomic importance of microconidial chains in *Fusarium* section *Liseola* and effects of water potential on their formation. Mycologia 75:693-698.

Fraedrich, S.W. and T. Miller. 1995. Mycoflora associated with slash-pine seeds from cones collected at seed orchards and cone-processing facilities in the south-eastern USA. European Journal of Forest Pathology 25:73-82.

Gordon, T.R. and D.Okamoto. 1992. Population structure and the relationship between pathogenic and nonpathogenic strains of *Fusarium oxysporum*. Phytopathology 82:73-77.

Guadet, J., J. Julien, J.F. Lafay and Y. Brygoo. 1989. Phylogeny of some *Fusarium* species, as determined by large-subunit rRNA sequence comparison. Molecular Biology and Evolution 6:227-242.

Headrick, J.M., J.K. Pataky and J.A. Juvik. 1990. Relationships among carbohydrate content of kernals, condition of silks after pollination, and the response of sweet corn inbred lines to infection of kernals by *Fusarium moniliforme*. Phytopathology 80:487-494.

Hildebrand, D.M. 1985. Pathogenicity of *Fusarium* spp. to lodgepole pine. USDA Forest Service, Rocky Mountain Region, Timber, Forest Pest and Cooperative Forestry Management. Biological Evaluation R2-85-8. 6p.

Huang, J.W. and E.G. Kuhlman. 1990. Fungi associated with damping-off of slash pine seedlings in Georgia. Plant Disease 74:27-30.

James, R.L. 1987. Occurrence of *Fusarium* on conifer tree seed from Northern Rocky Mountain nurseries. *In*: Landis, T.D. (tech. coord.). Proceedings: Combined Western Forest Nursery Council and Intermountain Nursery Association Meeting. USDA Forest Service, Rocky Mountain Forest & Range Experiment Station. General Technical Report RM-137. pp. 109-114.

James, R.L. 1991. Wilting of container-grown western white pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 123. 5p.

James, R.L. 1992. *Fusarium* sporodochia on container-grown ponderosa pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 126. 6p.

James, R.L. 1993. Fusarium species associated with post-emergence damping-off and root disease of young container-grown Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 129. 5p.

James, R.L. 1995. Root diseases of western white pine transplants - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Insect and Disease Management. Report 95-8. 10p.

James, R.L. 1996. Root disease of 1-0 bareroot seedlings - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 96-4. 10p.

James, R.L., R.K. Dumroese, C.J. Gilligan and D.L. Wenny. 1989. Pathogenicity of *Fusarium* isolates from Douglas-fir seed and container-grown seedlings. Idaho Forest, Wildlife and Range Experiment Station. Bulletin No. 52. 10p.

James, R.L., R.K. Dumorese and D.L. Wenny. 1988. Occurrence and persis- tence of *Fusarium* within styroblock and Ray Leach containers. *In*: Landis, T.D. (tech. coord.). Proceedings: Combined Meeting of the Western Forest Nursery Associations. USDA Forest Service, Rocky Mountain Forest & Range Experiment Station, General Technical Report RM-167. pp. 145-148.

James, R.L., R.K. Dumroese and D.L. Wenny. 1991. *Fusarium* diseases of conifer seedlings. *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada. Pacific and Yukon Region. Information Report BC-X-331. pp. 181-190.

James, R.L., R.K. Dumroese and D.L. Wenny. 1995. *Fusarium proliferatum* is a common, aggressive pathogen of container-grown conifer seedlings. Phytopathology 85:1129.

James, R.L., R.K. Dumroese and D.L. Wenny. 1997. Pathogenicity of *Fusarium proliferatum* in container-grown Douglas-fir seedlings. *In*: James, R. L. (ed.). Proceedings of the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). USDA Forest Service, Northern Region, Forest Health Protection. pp.

James, R.L., R.K. Dumroese, D.L. Wenny, J.F. Myers and C.J. Gilligan. 1987. Epidemiology of *Fusarium* on containerized Douglas-fir seedlings. 1. Seed and seedling infection, symptom production, and disease progression. USDA Forest Service, Northern Region, Forest Pest Management. Report 87-13. 22p.

James, R.L., D.S. Page-Dumroese, S.K. Kimball and S. Omi. 1996. Effects of *Brassica* cover crop, organic amendment, fallowing, and soil fumigation on production of bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 96-5. 16p.

James, R.L. and R.L. Woollen. 1988. An evaluation of the efficacy of hot water-chemical treatments to clean styroblock containers - Champion Timberlands Nursery, Plains, Montana. USDA Forest Service, Northern Region, Forest Pest Management. Report 89-5. 8p.

Jardine, D.J. and J.F. Leslie. 1992. Aggressiveness of *Gibberella fujikuroi* (*Fusarium moniliforme*) isolates to grain sorghum under greenhouse conditions. Plant Disease 76:897-900.

Joffe, A.Z. 1974. A modern system of *Fusarium* taxonomy. Mycopathologia et Mycologia applicata 53:201-228.

Kingsland, G.C. and C.C. Wernham. 1962. Etiology of stalk rots of corn in Pennsylvania. Phytopathology 52:519-523.

Klittich, C.J.R. and J.F. Leslie. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics 118:417-423.

Klittich, C.J.R. and J.F. Leslie. 1989. Chlorate-resistant, nitrate-utilizing (*crn*) mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Journal of General Microbiology 135:721-727.

Klittich, C.J.R. and J.F. Leslie. 1992. Identification of a second mating population within the *Fusarium moniliforme* anamorph of *Gibberella fujikuroi*. Mycologia 84:541-547.

Kuhlman, E.G. 1982. Varieties of *Gibberella fujikuroi* with anamorphs in *Fusarium* section *Liseola*. Mycologia 74:759-768.

Kuhlman, E.G., L.D. Dwinell, P.E. Nelson and C.Booth. 1978. Characterization of the *Fusarium* causing pitch canker of southern pines. Mycologia 70:1131-1143.

La Mondia, J.A. and W.H. Elmer. 1989. Pathogenicity and vegetative compatibility among isolates of *Fusarium oxysporum* and *F. moniliforme* colonizing asparagus tissues. Canadian Journal of Botany 67:2420-2424.

Leslie, J.F. 1991. Mating populations in *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology 81:1058-1060.

Leslie, J.F. 1995. *Gibberella fujikuroi*: available populations and variable traits. Canadian Journal of Botany 73(Supp. 1):S282-S291.

Leslie, J.F. and M.B. Dickman. 1991. Fate of DNA encoding hygromycin resistance after meiosis in transformed strains of *Gibberella fujikuroi* (*Fusarium moniliforme*). Applied and Environmental Microbiology 57:1423-1429.

Lim, G. 1967. *Fusarium* populations in rice field soils. Phytopathology 57:1152-1153.

Lodolo, E.J., W.H. Van Zyl and C.J. Rabie. 1992. A rapid molecular technique to distinguish *Fusarium* species. Mycological Research 97:345-346.

Logreico, A. and A. Bottalico. 1988. *Fusarium* species of the *Liseola* section associated with stalk and ear rot of maize in southern Italy, and their ability to produce moniliformin. Transactions of the British Mycological Society 90:215-219.

Logreico, A., A. Moretti, A. Ritieni, A. Bottalico and P. Corda. 1995. Occurrence and toxigenicity of *Fusarium proliferatum* from preharvest maize ear rot, and associated mycotoxins, in Italy. Plant Disease 79:727-731.

Magie, R.O. 1980. Fusarium disease of gladioli conrolled by inoculation of corms with nonpathogenic fusaria. Proceedings Florida State Horticultural Society 93:172-175.

Manicom, B.Q., M. Bar-Joseph, A. Rosner, H. Vigodsky-Haas and J.M. Kotze. 1987. Potential application of random DNA probes and restriction fragment length polymorphisms in the taxonomy of the fusaria. Phytopathology 77:669-672.

Marasas, W.F.O., N.P.J. Kriek, V.M. Wiggins, P.S. Steyn, D.K. Towers and T.J. Hastie. 1979. Incidence, geographic distribution, and toxigenicity of *Fusarium* species in South African corn. Phytopathology 69:1181-1185.

Marasas, W.F.O., P.E. Nelson and T.A. Toussoun. 1988. Reclassification of two important moniliformin-producing strains of *Fusarium*, NRRL 6022 and NRRL 6322. Mycologia 80:407-410.

Marasas, W.F.O., P.G. Thiel, C.J. Rabie, P.E. Nelson and T.A. Toussoun. 1986. Moniliformin production in *Fusarium* section *Liseola*. Mycologia 78:242-247.

Marin, S., V. Sanchis and N. Magan. 1995. Water activity, temperature, and pH effects on growth of *Fusarium moniliforme* and *Fusarium proliferatum* isolates from maize. Canadian Journal of Microbiology 41:1063-1070.

Marshall, M.R. and J.E. Partridge. 1981a. *Fusarium moniliforme* detection in corn (*Zea mays*) stalk tissue: identification of specific ribosomal proteins by polyacrylamide gel electrophoresis. Physiological Plant Pathology 18:133-141.

Marshall, M.R. and J.E. Partridge. 1981b. Immunochemical identification of *Fusarium moniliforme* ribosomes from diseased corn (*Zea mays*) stalk tissue. Physiological Plant Pathology 19:277-288.

Michailides, T.J. and D.P. Morgan. 1994. Dynamics of *Blastophaga psenes* populations, availability of caprifigs, and fig endosepsis caused by *Fusarium moniliforme*. Phytopathology 84:1254-1263.

Miller, J.D., M.E. Savard, A.Sibilia, S.Rapier, A.D. Hocking and J.I. Pitt. 1993. Production of fumonisins and fusarins by *Fusarium moniliforme* from Southeast Asia. Mycologia 85:385-391.

Msikita, W., P.E. Nelson, J.S. Yaninek, M. Ahounou and R. Fagbemissi. 1996. First report of *Fusarium moniliforme* causing cassava root, stem, and storage rot. Plant Disease 80:823.

Nava Saucedo, J.E., J.-N. Barbotin, M. Velut and D. Thomas. 1989. Ultrastructural examination of *Gibberella fujikuroi* mycelia: effect of immobilization in calcium alginate beads. Canadian Journal of Microbiology 35:1118-1131.

Nelson, P.E., L.W. Burgess and B.A. Summerell. 1990. Some morphological and physiological characters of *Fusarium* species in section *Liseola* and *Elegans* and similar species. Mycologia 82:99-106.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

Ocamb, C.M. and T. Kommedahl. 1994. Rhizosphere competence of *Fusarium* species colonizing corn roots. Phytopathology 84:166-172.

Ooka, J.J. and T. Kommedahl. 1977. Wind and rain dispersal of *Fusarium moniliforme* in corn fields. Phytopathology 67:1023-1026.

Peterson, S.W. 1991. Phylogenetic analysis of *Fusarium* species using ribosomal RNA sequence comparisons. Phytopathology 81:1051-1054.

Plattner, R.D., A.E. Desjardins, J.F. Leslie and P.E. Nelson. 1996. Identification and characterization of strains of *Gibberella fujikuroi* mating population A with rare fumonisin production phenotypes. Mycologia 88:416-424.

Puhalla, J.E. and P.T. Spieth. 1983. Heterokaryosis in *Fusarium moniliforme*. Experimental Mycology 7:328-335.

Puhalla, J.E. and P.T. Spieth. 1985. A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). Experimental Mycology 9:39-47.

Rowan, S.J. 1982. Tip dieback in southern pine nurseries. Plant Disease 66:258-259.

Schreuder, W., S.C. Lamprecht, W.F.O. Marasas and F.J. Calitz. 1995. Pathogenicity of three *Fusarium* species associated with asparagus decline in South Africa. Plant Disease 79:177-181.

Sidhu, G.S. 1983. Genetics of *Gibberella fujikuroi*. 1. Inheritance of certain cultural traits. Canadian Journal of Genetics and Cytology 25:93-96.

Snyder, W.C. and H.N. Hansen. 1945. The species concept in *Fusarium* with reference to *Discolor* and other sections. American Journal of Botany 32:657-666.

Storer, A.J., T.R. Gordon, D.L. Wood and P.L. Dallara. 1995. Pitch canker in California. California Dept. of Forestry and Fire Protection. California Forestry Note No. 110. 16p.

Subbarao, K.V. and T.J. Michailides. 1992. A re-evaluation of *Fusarium moniliforme* var. *fici*, the causal agent of fig endosepsis. Mycological Research 96:766-768.

Tiedt, L.R. and W.J. Jooste. 1988. Ultrastructure of collarette formation in *Fusarium* sect. *Liseola* and some taxonomic implications. Transactions of the British Mycological Society 90:531-536.

Tudzynski, B., K. Mende, K.-M. Weltring, J.R. Kinghorn and S.E. Unkles. 1996. The *Gibberella fujikuroi niaD* gene encoding nitrate reductase: isolation, sequence, homologous transformation and electrophoretic karyotype location. Microbiology 142:533-539.

Unestam, T., P. Chakravarty and E. Damm. 1987. Mycorrhizal protection of pine roots against pathogens. *In*: Sylvia, D.M., L.L. Hung and J.H. Graham (eds.). Proceedings 7th North American Conference on Mycorrhizae: Mycorrhizae in the Next Decade: Practical Applications & Research Priorities. University of Florida, Institute of Food and Agriculture Sciences. p. 267.

Van Asch, M.A.J., F.H.J. Rijkenberg and T.A. Coutinho. 1992. Phytotoxicity of fumonisin B1, moniliformin and T-2 toxin to corn callus cultures. Phytopathology 82:1330-1332.

Viljoen, A., M.J. Wingfield and W.F.O. Marasas. 1994. First report of *Fusarium subglutinans* f. sp. *pini* on pine seedlings in South Africa. Plant Disease 78:309-312.

Viljoen, A., M.J. Wingfield, W.F.O. Marasas and T.A. Coutinho. 1995. Characterization of *Fusarium* isolates from gladiolus corms pathogenic to pines. Plant Disease 79:1240-1244.

Waalwijk, C., J.R.A. de Koning, R.P. Baayen and W. Gams. 1996. Discordant grouping of *Fusarium* spp. from sections *Elegans*, *Liseola* and *Dlaminia* based on ribosomal ITS1 and ITS2 sequences. Mycologia 88:361-368.

Wagih, E.E., M.R.A. Shehata, S.A. Farag and M.K. Dawood. 1989. Dracena leaf proliferosis, a newly recorded disease affecting *Dracena sanderiana* in Egypt. Journal of Phytopathology 126:7-16.

Wall, G.C. and F.J. Cruz. 1991. *Lasiodiplodia theobromae* and *Fusarium proliferatum* causing storage rots of Taro on Guam. Plant Disease 75:1286.

Windels, C.E. 1991. Current status of *Fusarium* taxonomy. Phytopathology 81:1048-1051.

Xu, J.R., K. Yan, M.B. Dickman and J.F. Leslie. 1995. Electrophoretic karyotypes distinguish the biological species of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Molecular Plant-Microbe Interactions 8:74-84.

RECOVERY OF FUSARIUM SUBGLUTINANS F. SP. PINI FROM SHORTLEAF PINE CONES AND SEEDS[1]

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ABSTRACT

The contamination of cones and seeds of shortleaf pine by *Fusarium subglutinans* f. sp. *pini*, the pitch canker fungus, was studied at the USDA Forest Service Beech Creek Seed Orchard in 1995. *Fusarium subglutinans* f. sp. *pini* was isolated from the surface and interior of immature cones. However, recovery of the pitch canker fungus from immature cones was not indicative of contamination at the time of harvest. Isolation frequency of *F. subglutinans* f. sp. *pini* from the surface of shortleaf pine seed was a function of clones and sampling date. However, controlling the harvest date would not reduce contamination to acceptable levels. The fungus was recovered from an average of 61% of the untreated seeds. Only 1.6% of the shorleaf seeds were infested internally by the pitch canker pathogen. External contamination could possibly be eradicated by appropriate seed treatments.

INTRODUCTION

Pitch canker, caused by *Fusarium subglutinans* (Wollenweb. & Reinking) Nelson, Toussoun & Marasas f. sp. *pini* is an important disease of southern pines, including shortleaf pine (*Pinus echinata* Mill.)(Dwinell et al. 1985). Branch dieback in the upper crown, the predominant symptom of pitch canker on shortleaf pines, has been found in seed orchards, landscapes, progeny tests, and natural stands. *Fusarium subglutinans* f. sp. *pini* is also associated with the mortality of shorleaf pine seedlings in nurseries (S. Oak and M. Cram, personal communication) and is responsible for strobilus mortality and seed deterioration of southern pines (Miller and Bramlett 1979).

In the 1990s, pitch canker became a serious problem in the USDA Forest Service Beech Creek Seed Orchard near Murphy, North Carolina. In response, trees with severe pitch canker symptoms were removed. The quantity of shortleaf pine seeds is currently limited and USDA Forest Service (Region 8, Atanta, GA) wants to collect seed from the remaining trees during 1997. Because of seed problems and seedling mortality associated with shortleaf pine related to the pitch canker fungus, there is concern about the quality of the 1997 cone crop.

The objectives of this study were to determine: 1) if shortleaf pine cones and seeds are naturally contaminated or infected by the pitch canker fungus, 2) if the fungus is located within or on the seed surface, and 3) if time of cone collection affects seed quality.

MATERIALS AND METHODS

Immature cones:

In order to determine if *F. subglutinans* could be isolated from the surface or interior of immature shorleaf pine cones, 175 cones were collected at the Beech Creek Seed Orchard from a total of 35 trees (five cones/tree on September 7 and 27, 1995). Cones from each tree were placed in a plastic bag and maintained in a cooler for transport to the laboratory. In the laboratory, each cone was placed in a funnel, and washed with 10ml of sterile water. The wash water was collected in a test tube. A 1ml aliquot of the wash water was placed on a petri dish containing PCNB agar (Nash and Snyder 1962), a medium selective for *Fusarium* spp. The number of colony-forming units (CFU) per plate was determined after 10 days. Fifty randomly selected colonies were examined to confirm that the colonies were species of *Fusarium*. The colonies were transferred to KCl medium and examined microscopically when the cultures were 4- to 5-days-old for morphological characters of *F. subglutinans* and related species (Nelson et al. 1983).

External necrotic tissue was observed on 77 of the 175 cones; this necrosis was attributed mostly to insect damage. Samples of the necrotic tissue were excised from cones, flame-sterilized with 79%

[1] Paper presented at the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). Orlando-Gainesville, FL., USA. May 19-24, 1996.

[2] USDA Forest Service, Southern Research Station, Athens, GA, USA.

ETOH, and plated on PCNB agar. Colonies were examined to determine if the fungus was *F. subglutinans*.

The 175 cones were split vertically with a flame-sterilized knife, and five internal tissue samples were aseptically transferred to petri dishes containing PCNB agar. After 7-10 days, fungal colonies were examined to determine if they were *F. subglutinans*.

Mature cones:

Cones were collected from three trees of each of six clones (18 trees total) on October 18, 24, 30, and November 20, 1995. On each collection date, 25 cones were collected from throughout the crown of each tree. Cones were placed in plastic bags and maintained in a cooler for transport to the laboratory.

The specific gravity and moisture content were determined for five cones from each tree for each collection date (Oct. 18, 24 and 30) within 24 hours of harvesting. The specific gravity was not determined for the November 20 collection date because the cones had begun to open. Cone pedicils were cut flush with the cone surface and the cones were weighed. Cone specific gravity was determined using Barnett's procedure (Barnett 1979a). Cone moisture content was determined by drying the cones at 80°C for 48 hours and subsequently reweighing them. Cone moisture content was determined on a fresh weight and dry weight basis.

The remaining 20 cones from each tree were placed in 1/6 Kraft paper bags and stored in a ventilated building for 4-6 weeks. At the end of the storage period, cones were dried to a constant weight in a forced-air oven at 38°C for 48 hours. Seeds were extracted and stored at 4°C until they could be evaluated.

Seeds:

The percentage of potentially sound seeds was determined by using radiographic techniques (Bramlett et al. 1977). One hundred seeds from each tree at each collection date, except clone 40, were placed on Kodak Industrex 620 paper and exposed in a Hewlett Packard 4380N X-ray system. Because clone 40 did not produce enough seed on the first three sample dates for a full test, an average of 40 seeds were radiographed for each date.

Seed germination for each tree at each collection date was determined. Most test lots were replicated three times and each replicate consisted of 25 seeds. However, several seedlots did not yield enough seeds to meet this goal. Seeds were stratified using procedures outlined by Krugman and Jenkinson (1974). Seeds were placed in clear plastic containers that had been surface-sterilized with 0.5% sodium hypochlorite solution for 1 h. The bottoms of the containers were lined with sterile germination paper and moistened with sterile water. The number of germinated seeds for each seed lot was recorded after 7, 14, and 21 days.

Seeds from cones of each tree for each sampling date were evaluated for contamination by *F. subglutinans* and other potential fungal pathogens. Three evaluation procedures were used. In the first experiment, a sample of 25 seeds from each seedlot was placed on PCNB agar (five seeds per plate). In the second experiment, a sample of 25 seeds from each seedlot was surface-sterilized in 70% ETOH for 15 sec, followed by a 60 sec soak in 1% sodium hypochlorite. Seeds were blotted dry with sterile paper towels and aseptically cracked. The seeds were placed on PCNB agar (5 seeds per plate). In the third experiment, surface-sterilized seeds (25 from each seedlot) were not cracked before plating on PCNB agar. Seeds on PCNB agar were examined for the presence of fungi after 7-10 days.

Pathogenicity:

Pathogenicity tests were undertaken to confirm if *F. subglutinans* isolates from cones and seeds were the pitch canker pathotype (Dwinell et al. 1985). The shoots of 2-year-old Virginia pine (*P. virginiana* Mill.) growing under greenhouse conditions were inoculated with 20 randomly selected isolates of *F. subglutinans*. Isolates of the fungus were grown on potato dextrose agar for 7-10 days. Pine shoots were wounded with an inoculation needle and mycelium of the fungus placed in the wound with a sterile needle. Eight weeks after inoculations, shoot mortality resulting from girdling cankers was recorded.

RESULTS AND DISCUSSION

Immature cones:

Data for the two September sampling dates were pooled because they were drawn from non-normal populations. The nonparametric Mann-Whitney Rank Sum Test indicated that external and internal contamination between the two groups did not differ significantly. *Fusarium* spp. were not associated with 70 (40%) of the cones sampled from trees over the two sampling periods. An average of 321 CFU/cone of *Fusarium* spp. were obtained in the cone washes for the remaining 105 cones. *Fusarium sub-*

glutinans and *F. oxysporum* Schlecht. emend Snyd. & Hans. were the most frequently recovered species.

External necrotic tissue were observed on 77 of the immature shorleaf pine cones; these necrotic regions were caused primarily by insects. *Fusarium subglutinans* was isolated from 10.4% of the samples from these necrotic tissues.

Of the 175 cones sampled, *F. subglutinans* was recovered from the internal portions of 12%. The fungus was also present in the interior portions of about 50% of the cones from which *F. subglutinans* was recovered from the surface. The mode of entry of *F. subglutinans*, a wound parasite (Dwinell et al. 1985),is unknown.

Mature cones:

Cone moisture was a function of a highly significant interaction between clones and sampling date (two-way ANOVA).Percent cone moisture for clones 26, 40, and 8 was relatively stable over the sampling dates; however,percent cone moisture for clones 13, 28, and 2 declined an average on 9% (Table 1) between October 18 and October 30. Although not statistically significant, three clones (13, 28 and 2) with the lowest cone moisture content on October 30 (Table 1) were the same clones with the highest level of seed contamination (Table 2).

Cone specific gravity decreased from 1.0 on October 18 to an average of 0.96 on October 30. Cone specific gravity is often used as an indicator of cone and seed maturation for pine species. Krugman and Jenkinson (1974) recommend collecting shortleaf pine cones when specific gravity decreases to below 0.89. However, the present study revealed no relationship between specific gravity of shortleaf pine cones and fungal contamination of cones and seeds. In addition, although Barnett's technique (1979a, 1979b) is useful for measuring specific gravity of longleaf (*P. palustris* Mill.), slash (*P. elliottii* Engelm. var. *elliottii*) and loblolly (*P. taeda* L.) pine cones, it has some shortcomings when applied to shortleaf pine cones. Essentially, the specific gravity of shortleaf pine cones cannot be used to determine when the cones should be harvested.

Seeds:

Isolation of *F. subglutinans* from untreated shorleaf pine seed was related to a statistically significant clone x sampling date interaction (Table 2). The rate of *F. subglutinans* isolation from seeds for clones 13 and 28 ranged from 87-100% among the four sampling dates. Level of contamination for clone 2 increased significantly between October 18 and Oct 24. Isolation of *F. subglutinans* was usually less and more variable from seed of clones 26, 40, and 8 than from clones 13, 28, and 2. *Fusarium subglutinans* was isolated from 61% of the untreated shorleaf pine seed among all clones and sampling dates.

Table 1. Shortleaf pine cone moisture content (fresh weight basis) as a function of interactions between clones and sampling dates.

	Sampling Dates (1995)[1]			
Clone	October 18 (%)	October 24 (%)	October 30 (%)	Mean (%)
13	50.5a	48.3b	45.8b	49.6
28	56.6a	51.4b	46.7c	51.6
2	56.5a	48.9b	43.4c	49.6
26	53.8a	52.4a	55.8a	54.0
40	58.7a	56.9a	59.2a	58.3
8	54.0a	52.8a	54.1a	53.7
Mean	55.0	51.8	50.8	52.6

[1] Within each row, percentages followed by the same letter are not significantly different (P=0.05) according to the Tukey test.

Table 2. Isolation of *Fusarium subglutinans* from non-surface sterilized shortleaf pine seed as a function of interactions between clones and sampling dates.

	Sampling Dates (1995)[1]				
Clone	October 18 (Percent)	October 24 (Percent)	October 30 (Percent)	November 20 (Percent)	Mean (Percent)
13	100a	100a	100a	100a	100
28	87a	99a	100a	96a	95
2	13b	88a	96a	95a	73
26	0c	56b	94a	64b	53
40	0c	27b	33ab	48a	27
8	15b	18b	37a	12b	20
Mean	36	64	77	69	61

[1] Within each row, percentages followed by the same letter are not significantly different (P=0.05) according to the Tukey test.

Fusarium subglutinans was isolated very infrequently from cracked or noncracked shortleaf pine seeds that had been surface-sterilized (Table 3). These infrequent isolations may represent true seed infection or fungal contamination not eliminated by surface sterilization. In either case, based on these experiments, most *F. subglutinans* contamination appears to be superficially located on the seedcoat of shorleaf pine, not internally within the endosperm and embryo.

Because the number of seeds for each seedlot was insufficient for all germination tests, the data were analyzed by two-way ANOVA for clone and sampling dates without interaction. The experimental variation was primarily due to clones (P=0.026); therefore, the data were pooled by clones (Table 4). Germination ranged from 32-73%. Futhermore, two-way ANOVA of radiographic data revealed that clonal variation occurred in the%age of filled seeds. However, mean separation procedures revealed that this clonal variation was primarily due to the differences between clone 8 and 28 (Table 4). No correlation appeared between germination percentage and percentage of filled seeds (R=0.120). If the seedlots were pooled and the empty seeds removed, an overall germination rate of these seeds would probably be approximately 81%.

Pathogenicity:

About 80% of the *F. subglutinans* isolated from shortleaf pine cones and seeds were pathogenic to Virginia pine by causing shoot mortality. Therefore, most of the *F. subglutinans* isolated in this study were probably the pitch canker pathotype.

Table 3. Isolation of *Fusarium subglutinans* from shortleaf pine seed that had been surface-sterilized and either noncracked or cracked before plating on *Fusarium*-selective media.

	Seed Treatment[1]	
Clone	Cracked (Percent)	Noncracked (Percent)
13	4.3	4.0
28	3.0	2.6
2	1.0	1.3
26	0	0.7
40	1.3	1.3
8	0	0
Mean	1.6	1.4

[1] Based on an average of 77 seeds/clone/sample period. Treatment means are not significantly different.

CONCLUSIONS

The pitch canker fungus, *F. subglutinans* f. sp. *pini*, was isolated from the surface and interior of immature shortleaf pine cones harvested at the seed orchard. However, isolation of the fungus from immature cones was not indicative of seed contamination at the time of harvest. Isolation frequency from the surface of seeds was a function of clone and sampling date. Controlling sampling date would not reduce contamination to acceptable levels. Furthermore, without several years' data, it cannot be concluded that clones with the highest levels of external seed contamination in 1995 would rank the same in subsequent years.

Only 1.6% of the shorleaf pine seeds were infected internally, and external contamination could possibly be eradicated by appropriate seed treatments without adverse impact on seed germination. Soaking shortleaf pine seeds in 30% solution of hydrogen peroxide for 15 min. has shown promise for surface decontamination (Barnett 1976). At present, the relationship between the surface contamination of pine seeds by *F. subglutinans* and pre- and post-emergence damping-off as well as disease in older seedlings has not been fully evaluated. Seed treatments represent the best insurance to prevent potential seedling losses.

Table 4. Radiography and germination data for shortleaf pine seeds extracted from cones collected at the USDA Forest Service Beech Creek Seed Orchard.

Clone	Germination[1] (Percent)	Filled Seed[1] (Percent)
13	72.5a	75.1ab
28	44.0ab	62.9b
2	31.8b	69.8ab
26	56.8ab	71.1ab
40	46.2ab	71.3ab
8	67.5ab	82.9a
Mean	53.1	72.2

[1] Data were pooled across ramets and sampling periods. Within each column, percentages followed by the same letters are not significantly different (P=0.05) according to the Tukey test.

LITERATURE CITED

Barnett, J.P. 1976. Sterilizing southern pine seeds with peroxide. Tree Planters' Notes 27:17-19.

Barnett, J.P. 1979a. An easy way to measure cone specific gravity. *In*: Seed Collection: Proceedings of the Workshop, May 16-18, 1979, Macon, GA. USDA Forest Service, Technical Publication. SA-TP 8. pp. 21-23.

Barnett, J.P. 1979b. Southern pine cone maturation and storage. *In*: Seed Collection: Proceedings of the Workshop, May 16-18, 1979, Macon, GA. USDA Forest Service, Technical Publication. SA-TP 8. pp. 11-20.

Bramlett, D.L., E.W. Belcher, Jr., G.L. DeBarr, G.D. Hertel, R.P. Karrfalt, C.W. Lantz, T. Miller, K.D. Ware and H.O. Yates III. 1977. Cone analysis of southern pines: a guidebook. USDA Forest Service, General Technical Report. SE-13. 28p.

Dwinell, L.D., J.B. Barrow-Broaddus and E.G. Kuhlman. 1985. Pitch canker: a disease complex of southern pines. Plant Disease 69:270-276.

Krugman, S.L. and J.L. Jenkinson. 1974. Seed testing. *In*: Schopmeyer, C.S. (tech. coord.). Seeds of Woody Plants in the United States. USDA Agricultural Handbook 450. pp. 598-638.

Miller, T. and D.L. Bramlett. 1979. Damage to reproductive structures of slash pine by two seedborne pathogens: *Diplodia gossypina* and *Fusarium moniliforme* var. *subglutinans*. *In*: Flowering and Seed Development in Trees: symposium proceedings. USDA Forest Service, Southern Forest Experiment Station. pp. 347-355.

Nash, S.M. and W.C. Snyder. 1962. Quantitative estimations by plate counts of propagules of the bean rot *Fusarium* in field soils. Phytopathology 52:567-572.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

MORTALITY OF LONGLEAF PINE SEEDLINGS CAUSED BY FUSARIUM SUBGLUTINANS AND AN EVALUATION OF POTENTIAL INOCULUM SOURCES[1]

S.W. Fraedrich[2] and L.D. Dwinell[2]

ABSTRACT

Mortality of longleaf pine seedlings due to *Fusarium subglutinans* and other *Fusarium* spp. has been a periodic problem at bareroot and container nurseries in the southern United States. Seedling mortality has been observed during site visits at one nursery since 1994 and *F. subglutinans* was consistently isolated from dead and dying seedlings. Possible inoculum sources of *F. subglutinans* were evaluated at this nursery. *Fusarium subglutinans* was isolated from seeds of seedlots sown at the nursery; frequency of isolation ranged from 2-15 percent among seedlots. The fungus was also detected in spore traps placed beneath pine trees that border the nursery. *Fusarium subglutinans* was isolated from a high percentage of diseased and healthy-appearing seedlings, but not from the pine bark mulch used at the nursery. *Fusarium subglutinans* is a wound pathogen of longleaf pine seedlings. All tested isolates of *F. subglutinans* were pathogenic to longleaf pine seedlings.

INTRODUCTION

Longleaf pine (*Pinus palustris* Mill.) was once a dominate species that occurred throughout the southern United States. According to Wahlenberg (1946), longleaf pine may have occupied as much as 24 million hectares (60 million acres) at one time, but by 1989, the area had declined to only 1.5 million hectares (3.8 million acres)(Kelly and Bechtold 1990). Presently, longleaf pine occupies only 3 percent of the total timberland in the South (Kelly and Bechtold 1990).

In recent years, there has been renewed interest in reestablishment of longleaf pine on sites formerly occupied by this species. Many of the previous difficulties that prevented adequate artificial and natural regeneration of long eaf pine have been overcome with development of new techniques and acquisition of knowledge about the biology of this species (Barnett et al. 1990; Boyer and White 1990). In addition, the longleaf pine forest type is recognized as being unique among southern pine ecosystems, and reforestation with longleaf pine is being promoted by many land management agencies and organizations interested in species conservation.

Seed-borne contamination by *Fusarium subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas f. sp. *pini* and mortality of seedlings caused by this fungus have been problems for managers of longleaf pine nurseries (Carey and Kelley 1994; Runion and Bruck 1988). At one nursery, *F. subglutinans* has been consistently isolated from diseased longleaf pine seedlings during the last three growing seasons, and the nursery manager has observed this problem since 1989. The disease was partially responsible for severely understocked nursery beds. At another nursery, mortality of 7-month-old longleaf pine seedlings caused by *F. subglutinans* was restricted primarily to seedlings produced from one particular seedlot. Seedlings grown from other seedlots in adjacent nursery beds were unaffected by the disease (Fraedrich, unpublished), suggesting that seeds may have been a primary source of inoculum.

The present study was undertaken to evaluate potential inoculum sources of *F. subglutinans* in one longleaf pine bareroot nursery that has experienced disease-related losses during the past several years.

MATERIALS AND METHODS

Seedling mortality:

The study was conducted at the USDA Forest Service Ashe Nursery, located near Brooklyn, MS. Evaluations of disease in longleaf pine nursery beds were initiated in February or March of 1995 and 1996, when seedlings were approximately 4-5 months old. These evaluations continued through to the lifting of

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seedlings. During site visits, seedlings in various stages of disease were collected throughout nursery beds and transported to the laboratory for observations and to determine the causal agent.

A selective agar medium, developed by Nash and Snyder (1962) was used to isolate *Fusarium* spp. The procedures and taxonomic keys described by Nelson et al. (1983) were used to identify *Fusarium* spp. Identifications were made primarily on carnation leaf (CLA) and KCl agar media.

Evaluation of potential inoculum sources:

Seedborne Fusarium spp.:

Five seedlots used at the nursery were evaluated for presence of *Fusarium* spp. For each seedlot, 50-200 seeds were assessed. Seeds were rinsed in 70 percent ethyl alcohol for 15 seconds and placed in a 0.5 percent-1.0 percent sodium hypochlorite solution for 1-2 min. Seeds were rinsed 3 times with sterile distilled water, blot-dried, and plated on the Nash-Snyder selective agar medium. After 5-10 days, the number of seeds positive for *Fusarium* spp. was recorded. All *Fusarium* spp. were transferred to KCl and CLA media during initial evaluations of some seedlots; isolates were subsequently identified to species. In later evaluations, only *Fusarium* isolates with colony morphology similar to *F. subglutinans* were transferred and identified.

Airborne Fusarium spp.:

Evaluations of airborne *Fusarium* spp. were conducted during several visits to the nursery. Spore traps, which consisted of petri dishes with the Nash and Snyder agar medium, were placed on stands for approximately 12 hours during the night. The stands were approximately 1.2m high and placed in the middle and edges of nursery beds, as well as under trees that bordered the nursery. Three plates were placed on each stand during each sampling period. Plates were returned to the laboratory, incubated for 7-10 days at 25°C, and counts made of the number of *Fusarium* colonies. Isolates of *Fusarium* were transferred and identified as described above.

Assessment of mulch for Fusarium spp.:

Samples of bark and wood chips used as mulch were collected at two sampling times in the spring of 1995 from six storage piles located at various places throughout the nursery. In the first sampling, a 25g sample from each pile was placed in 200ml of sterile distilled water and stirred for several minutes with a magnetic stirrer. One ml aliquots from each sample were placed on each of three petri dishes containing the Nash-Snyder agar medium. Plates were incubated for 7-10 days at 25°C and the number of *Fusarium* colonies were counted. During the second sampling, a 4g sample of the mulch from each pile was placed in 200ml of sterile distilled water, and dilutions were subsequently made to 1:100, 1:200, and 1:400 g/ml. One ml aliquots of each dilution from each sample were placed on each of three petri dishes containing the Nash-Snyder agar, and incubated and counted as previously described. Isolates of *Fusarium* spp. with colonly morphology similar to that of *F. subglutinans* on Nash-Snyder media were transferred and identified.

Assessment of nursery soil for Fusarium spp.:

In March 1995, two soil samples were collected from each of three fields in the nursery. Each sample was a composite of 10-15 soil cores, collected to a 15cm depth, and obtained at random locations throughout the field. One field was in production for longleaf pine seedlings at the time of evaluation. The second field had been in production until January 1995 when the seedlings were lifted. The third field was in cover crops and had not been in production in recent years. Soil samples were thoroughly mixed. A 10g subsample of each sample was placed in 200ml of sterile distilled water and stirred for several minutes with a magnetic stirrer. Dilutions were subsequently made to 1:200 and 1:400 g/ml. One ml aliquots of each dilution were then palced on each of five petri dishes containing the Nash-Snyder medium. Plates were incubated for 7-10 days at 25°C. *Fusarium* colonies were counted, and randomly selected isolates of *Fusarium* were transferred and identified.

Association of F. subglutinans with healthy and diseased seedlings:

The association of *F. subglutinans* with healthy-appearing and diseased seedlings was assessed in June and August 1995. Thirteen longleaf pine seedlings exhibiting disease symptoms were located in nursery beds during the June sampling period.

Dead seedlings were lifted from nursery beds, placed individually in plastic bags, and labeled. Three healthy-appearing seedlings adjacent to each dead seedling were also lifted and placed in plastic bags. In the laboratory, roots of diseased and healthy-appearing seedlings were removed at approximately 1.3cm below the root collar. Needles were clipped to within 0.65cm of the needle base. The remaining portion of seedlings were rinsed

thoroughly with running tap water to remove all loose soil and placed individually in a test tube with 25ml of sterile distilled water. Test tubes with seedlings were placed on a vortex mixer for 30 sec. Seedlings were removed from test tubes and 1ml aliquots of the wash water were placed on each of three petri dishes with Nash-Snyder agar. The wash water was spread with a sterile, bent glass rod and plates were incubated for 7-10 days at 25°C. Isolates of representative *Fusarium* colonies were transferred and identified. The August sampling was conducted in a similar manner except that one-three seedlings exhibiting disease symptoms were collected at each of seven disase spots in nursery beds (14 seedlings total). Three healthy-appearing seedlings were collected adjacent to dead seedlings in each of the seven spots (21 seedlings total).

Pathogenicity tests:

Several experiments were conducted to assess the pathogenicity of *F. subglutinans* isolates from the various sources. Isolates selected for pathogenicity tests were single spored. One experiment was designed to determine whether *F. subglutinans* was a wound pathogen on 6-week-old longleaf pine seedlings. Four isolates of *F. subglutinans* were grown on potato dextrose agar (PDA) medium for 7-14 days at 25°C. Conidia of each isolate were separated from hyphae by placing 25-30ml of sterile distilled water on petri dishes, rubbing the mycelial mats with a glass rod and passing the suspension through sterile cheesecloth. Spore suspensions were calibrated to 10^6 spores/ml. For each fungal isolate, 15-22 seedlings were punctured with an inoculation needle and subsequently sprayed with conidia. Another 15-22 seedlings for each fungal isolate were not wounded, but were sprayed with conidial suspensions. Sets of wounded and nonwounded seedlings, sprayed with sterile distilled water, served as controls. All seedlings were placed in a growth chamber at 25°C and observed every 2-4 days.

Other pathogenicity tests were conducted by growing isolates of *F. subglutinans* on PDA for 7-14 days and then inserting a small amount of mycelium into puncture wounds on seedling stems. Five 6- to 8-month-old longleaf pine seedlings were used for each isolate of *F. subglutinans*. Seedlings were observed every 2-4 days and the number of dead seedlings was recorded after 8-10 weeks. Pathogenicity tests were conducted on 20 isolates of *F. subglutinans*.

RESULTS

Seedling mortality:

Foliage of diseased seedlings typically wilted and often turned brown with a darker discoloration developing toward the inner portions of needles closest to the stem. Necrotic tissues indicative of an infection were often observed in the root collar region of seedlings and necrosis seemed to extend downward in the tap root over time. Roots of diseased seedlings often appeared alive even though the tops may have wilted and appeared dead. Other causes of mortality were also observed, and included freeze and herbicide damage.

Evaluation of potential inoculum sources:

Seedborne Fusarium spp.:

Fusarium spp. were isolated from 16-50 percent of the seeds from five longleaf pine seedlots. *Fusarium subglutinans* was isolated from 2-15 percent of the seeds among seedlots. Other *Fusarium* spp. often associatied with these seeds included *F. oxysporum* Schlecht., *F. proliferatum* (Matsushima) Nirenberg, and *F. moniliforme* Sheld.

Airborne Fusarium spp.:

Fusarium spp. were primarily detected in spore traps during nights when rain occurred. Results of sampling periods for March 29 and 30, 1995 are presented in Table 1. Rain occurred during each of the two nights. Spore traps located in nursery beds had relatively few colony forming units (CFU) of *Fusarium* spp. on either night. In contrast, CFU of *Fusarium* spp. were frequently recovered in spore traps located under pine trees that bordered the nursery. *Fusarium subglutinans* was among the species routinely detected on spore traps located under trees; only one CFU of *F. subglutinans* was detected on traps from the nursery beds. Other *Fusarium* spp. that were isolated included *F. oxysporum*, *F. solani* (Mart.) Sacc., and *F. chlamydosporum* Wollenw. & Reinking.

Assessment of mulch for Fusarium spp.:

Fusarium spp. were isolated infrequently from the wood- and bark-chip mulch for both sampling periods. Growth of bacteria and fungi other than *Fusarium* spp. on the selective medium was often a prob-

lem, and competition from other organisms may have interferred with the recovery of *Fusarium* spp. *Fusarium* spp. isolated included *F. oxysporum*, *F. proliferatum*, and *F. moniliforme*. *Fusarium subglutinans* was not isolated from these samples.

Table 1. Isolation of *Fusarium subglutinans* and other *Fusarium* spp. from spore traps located in the nursery and under pine trees that border the nursery on March 29 and 30, 1995.

Station	Location	Mean Number of *Fusarium* Colonies Per Plate (3/29)	Mean Number of *Fusarium* Colonies Per Plate (3/30)	*Fusarium subglutinans* Present
1	Field	1	0	No
2	Field	0	0	No
3	Field	0	0	No
4	Field	0	1	No
5	Field	0	0	No
6	Field	1	0	Yes
7	Forest	31	49	Yes
8	Field	0	0	No
9	Forest	14	20	Yes
10	Forest	2	3	Yes
11	Forest	1	36	Yes

Assessment of nursery soil for Fusarium spp.:

Estimates of *Fusarium* populations in soil ranged from approximately 575 CFU/g dry weight of soil in the field currently in longleaf pine production to 2400 CFU/g in the field where longleaf pine was recently lifted. The estimate for the field in cover crops was approximately 940 CFU/g, although considerable variation was observed among samples for this field. *Fusarium subglutinans* was isolated from field soil that was currently in longleaf pine production, but not from fields that were not in production. Other *Fusarium* spp. isolated from soil samples included *F. oxysporum*, *F. solani*, *F. proliferatum* and *F. chlamydosporum*.

Association of F. subglutinans with healthy and diseased seedlings:

Fusarium subglutinans was isolated from the wash water of both healthy-appearing and diseased longleaf pine seedlings (Figure 1). Colony-forming units of *F. subglutinans* were isolated from the wash water of more than 90 percent of the healthy-appearing and diseased seedlings in the June sampling. In August, the isolation frequency from both healthy-appearing and diseased seedlings was less than in June. *Fusarium proliferatum* was isolated frequently from seedlings in the August sampling.

Pathogenicity tests:

All isolates of *F. subglutinans*, tested thus far, from healthy-appearing and diseased seedlings, seeds, and spore traps were pathogenic to longleaf pine seedlings. Seedlings which were inoculated with isolates of *F. subglutinans* developed characteristic symptoms of disease and subsequently died. Wounded, non-inoculated seedlings generally remained healthy and failed to develop characteristic disease symptoms. Isolates of *F. subglutinans* and other *Fusarium* spp. from various sources will continue to be screened for pathogenicity. *Fusarium subglutinans* is a wound pathogen of longleaf pine seedlings (Table 2). All wounded, inoculated seedlings died. Seedling mortality occurred at very low levels in control seedlings and in nonwounded, inoculated seedlings. Mortality of "control" seedlings in this experiment was primarily caused by insects.

DISCUSSION

Mortality of longleaf pine seedlings caused by *F. subglutinans* f. sp. *pini* represents a potential problem for the production of nursery stock in the southern United States. Seedborne contamination is believed to be a primary source of inoculum for some seedling diseases in conifer nurseries (Graham and Linderman 1983; Pawuk 1978; Runion and

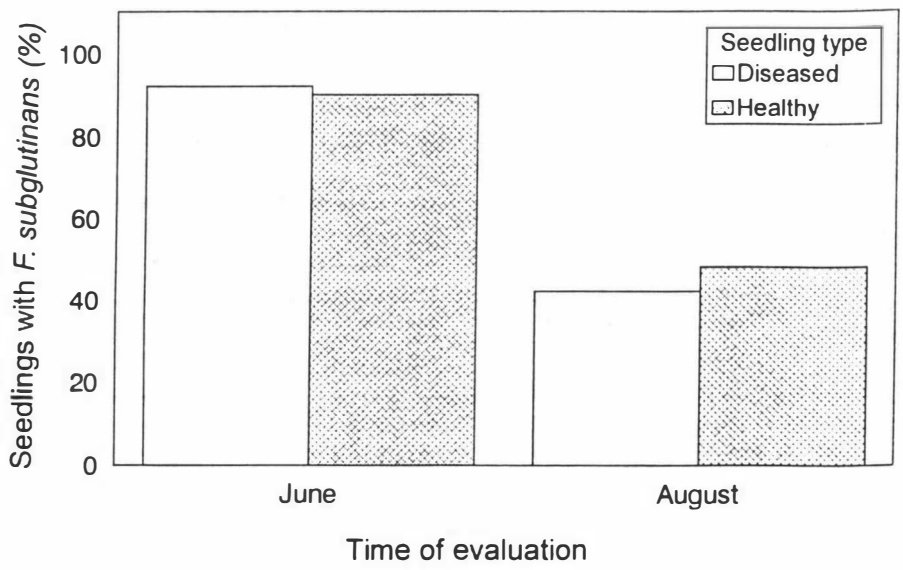

Figure 1. Percentage of healthy-appearing and diseased seedlings from which *Fusarium subglutinans* was isolated in seedling washings when sampled in June and August, 1995.

Bruck 1988). *Fusarium subglutinans* was isolated from seeds of seedlots used at the nursery, and seeds certainly represent a potential avenue by which the fungus could become established in nursery beds. However, seedborne inoculum does not represent the only potential inoculum source of this fungus. Pine trees bordering the nursery may also be an inoculum source from which *F. subglutinans* could become established in longleaf pine seedling crops. In addition, *F. subglutinans* may spread from diseased seedlings to neighboring healthy seedlings. Because longleaf pines are fall sown and grown in nursery beds for approximately 15-16 months, inoculum may be produced on dead and infected seedlings in the older plantings at a time when a new longleaf pine crop is being established year. Determination of the potential for spread of inoculum from older seedling crops to newly established crops requires additional investigation.

A pine bark mulch is used in some nurseries following sowing. The nursery manager at the study site was concerned that the bark and wood chips making up the mulch may be contaminated by *F. subglutinans*. However, no evidence has been found to support this concern. Likewise, *F. subglutinans* was not isolated from soil of fields that were no longer in longleaf pine production, although the fungus was recovered from a field with longleaf pine seedlings present at the time of sampling. *Fusarium subglutinans* is not regarded as a competitive soilborne fungus. Bolkan et al. (1979) found that the species was short-lived in soil, surviving for only 6-13 weeks in the absence of host tissues. The potential for survival of *F. subglutinans* in soil from one seedling crop to the next seems unlikely.

All isolates of *F. subglutinans* tested to date were capable of causing disease on longleaf pine seedlings, and therefore, can be regarded as the *F. subglutinans* f. sp. *pini* pathotype proposed by Correll et al (1991). Results of this study indicated that *F. subglutinans* was also a wound pathogen of longleaf pine as found for other southern pine species (Dwinell et al. 1985; Hepting and Roth 1946; Kuhlman 1987). At present, information about the wounding agents that occur naturally in longleaf pine nurseries is limited.

Results of this study indicated that in addition to seeds, other potential inoculum sources of *F. subglutinans* may exist in longleaf pine nurseries. The importance of specific sources of inoculum in development of this seedling disease in southern pine nurseries is unknown. At present, little is known about inoculum spread and wounding agents that may influence disease development in longleaf pine seedlings. Better information on the epidemiology of this seedling disease is necessary in order to

develop appropriate integrated pest management practices for nursery managers.

Table 2. Results of pathogenicity tests with four isolates of *Fusarium subglutinans* on wounded and non-wounded 6-week-old longleaf pine seedlings.

Isolate	Isolate Source	Wound Treatment	No. Seedlings Inoculated	No. Seedlings Dead	Percent Dead
8H2-3	Healthy Seedling	None Wounded	20 17	1 17	5 100
10H2-4	Healthy Seedling	None Wounded	15 18	0 18	0 100
A1	Diseased Seedling	None Wounded	21 23	3 23	14 100
ST1	Spore Trap	None Wounded	19 22	4 22	21 100
Control		None Wounded	20 20	4 2	20 10

LITERATURE CITED

Barnett. J.P., D.K. Lauer and J.C. Brissette. 1990. Regenerating longleaf pine with artificial methods. *In*: Farrar, R.M., Jr. (ed.). Proceedings of the Symposium on the Management of Longleaf Pine. USDA Forest Service, General Technical Report SO-75. pp. 72-93.

Bolkan, H.A., J.C. Dianese and F.P. Cupertino. 1979. Survival and colonization potential of *Fusarium moniliforme* var. *subglutinans* in soil. Phytopathology 69:1298-1301.

Boyer, W.D. and J.B. White. 1990. Natural regeneration of longleaf pine. *In*: Farrar, R.M., Jr. (ed.). Proceedings of the Symposium on the Management of Longleaf Pine. USDA Forest Service, General Technical Report SO-75. pp. 93-113.

Carey, W.A. and W.D. Kelley. 1994. First report of *Fusarium subglutinans* as a cause of late-season mortality in longleaf pine nurseries. Plant Disease 78:754.

Correll, J.C., T.R. Gordon, A.H. McCain, J.W. Fox, C.S. Koehler, D.L. Wood and M.E. Schultz. 1991. Pitch canker disease in California: pathogenicity, distribution, and canker development on Monterey pine (*Pinus radiata*). Plant Disease 75:676-682.

Dwinell, L.D., J. Barrows-Broaddus and E.G. Kuhlman. 1985. Pitch canker: a disease complex. Plant Disease 69:270-276.

Graham, J.G. and R.G. Linderman. 1983. Pathogenic seedborne *Fusarium oxysporum* from Douglas-fir. Plant Disease 67:323-325.

Hepting, G. and L.F. Roth. 1946. Pitch canker, a new disease of some southern pines. Journal of Forestry 44:742-744.

Kelly, J.F. and W.A. Bechtold. 1990. The longleaf pine resouce. *In*: Farrar, R.M., Jr. (ed.). Proceedings of the Symposium on the Management of Longleaf Pine. USDA Forest Service, General Technical Report SO-75. pp. 11-22.

Kuhlman, E.G. 1987. Effects of inoculation treatment with *Fusarium moniliforme* var. *subglutinans* on dieback of loblolly and slash pine seedlings. Plant Disease 71:161-162.

Nash, S. and W.C. Snyder. 1962. Quantitative estimation by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennyslvania State University Press, University Park. 193p.

Pawuk, W.H. 1978. Damping-off of container-grown longleaf pine seedlings by seed borne Fusaria. Plant Disease Reporter 62:82-84.

Runion, G.B. and R.I. Bruck. 1988. Effects of thiabendazole-DMSO treatment on longleaf pine seed contaminated with *Fusarium subglutinans* on germination and seedling survival. Plant Disease 72:872-874.

Wahlenberg, W.G. 1946. Longleaf pine: its use, ecology, regeneration, protection, growth and management. Charles Lathrop Pack Forestry Foundation and USDA Forest Service. Washington, DC. 429p.

DEVELOPMENT OF DNA DIAGNOSTICS FOR DETECTING FUSARIUM IN CONIFER NURSERY ENVIRONMENTS[1]

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ABSTRACT

Our goal was to develop a DNA-based system for differentiating *Fusarium* species commonly associated with conifer seedlings in bareroot forest nurseries. We also wanted to characterize tissue and soil isolates and locate genetic markers for pathogenicity in *F. oxysporum*, an important root disease pathogen of conifer seedlings, using molecular techniques. Our approach was a combination of pathogenicity testing and molecular methods. Blind testing indicated approximately 90 percent accuracy in identifying *Fusarium* species using restriction fragment length polymorphisms (RFLPs). This approach has promise for use with environmental samples such as soil and conifer tissues. Another molecular technique, amplified fragment length polymorphism (AFLP) analysis, provided intraspecific resolution and preliminary results indicated a significant difference between soil and tissue populations of *F. oxysporum*. This method will be used to search for pathogenicity or virulence markers, pending completion of additional pathogenicity tests.

INTRODUCTION

In bareroot conifer nurseries of the northwestern United States, *Fusarium* species are responsible for considerable losses due to pre- and post-emergence damping-off and root diseases. Methyl bromide is often the treatment of choice for control of *Fusarium* diseases as well as several other soil-borne pathogens, but has fallen into disfavour because it is an ozone depleting chemical. Under the Clean Air Act, the U.S. Environmental Protection Agency has prohibited the production and importation of methyl bromide starting January 1, 2001. This work aims to develop new monitoring technologies to assess the efficacy of alternative control measures. *Fusarium* species are particularly difficult to identify microscopically and may require considerable time and expertise. Furthermore, while some isolates are virulent pathogens, others are harmless soil saprophytes. Restriction fragment length polymorphisms (RFLPs) were previously able to differentiate *Fusarium* species including *F. oxysporum* Schlecht., *F. proliferatum* (Matsushima) Nirenberg, *F. moniliforme* Sheldon, *F. avenaceum* (Fr.) Sacc., and *F. acuminatum* Ell. & Ev. (Donaldson et al. 1995). The major goals of our work were to develop a DNA-based system for differentiating *Fusarium* species commonly associated with conifers, use molecular methods to characterize tissue and soil isolates of *F. oxysporum*, and to locate genetic markers for pathogenicity in *F. oxysporum* isolated from conifers.

MATERIALS AND METHODS

Two molecular methods used in this study relied on polymerase chain reaction (PCR) technology (for reviews see Arnheim and Erlich 1992; Foster et al. 1993; Henson and French 1993). The first method was RFLP (Sambrook et al. 1989) analysis of the histone H3 gene (Donaldson et al. 1995; Glass and Donaldson 1995). The first step in our RFLP analysis was amplification of the histone H3 target by means of PCR. Next, each amplification product was digested using four different restriction endonucleases which recognize and cut the amplified DNA at specific sites. Resulting DNA fragments were then separated by agarose gel electrophoresis and visualized under ultraviolet light after staining with ethidium bromide. Each restriction endonuclease digestion pattern was then recorded as a single letter code (e.g. A, B, or C). When combined, the four restriction digestion patterns formed a four letter code (e.g. BUCD) which was interpreted as an allele of the H3 gene. Frequencies of these alleles in different groups of isolates were compared using the Chi-square statistic available in SYSTAT(for Windows, Version 6.0, SPSS Inc., Chicago, IL).

A second molecular technique known as amplified fragment length polymorphism (AFLP) analysis (Za-

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beau and Vos 1993) was used to find additional intraspecific variation in *F. oxysporum* isolates. This technique relied on the ligation of a double-stranded DNA adapter to genomic DNA which had been treated with a restriction endonuclease. By using primers which were complementary to the adapter sequence, the polymerase chain reaction amplified these restriction fragments. By adding one to three additional bases to the primer, a subset of the total restriction fragment population was amplified. Amplification products were separated by agarose gel electrophoresis and visualized as above. The banding pattern produced by each amplification was again recorded using a single letter code. As with the H3-RFLP data, comparisons between different groups were made using the Chi-square statistic to determine if significant differences were present based on AFLP haplotype frequencies.

Pathogenicity tests were done using a method similar to the assay described by Axelrood et al. (1995). Douglas-fir seeds were stratified for 3 weeks and surface sanitized immediately before use in 30 percent hydrogen peroxide for 1 hour. After rinsing the seeds eight times with sterile distilled water, they were placed in the appropriate inoculum and allowed to soak for 10 minutes. Inocula were prepared by growing *Fusarium* isolates on potato dextrose agar (PDA) for 1 week and preparing spore suspensions containing approximately 1 X 10^5 spores/ml. This was determined prior to the start of the assay using a haemocytometer and confirmed by dilution plating. After soaking, seeds were placed into GA-7 jars (Magenta Corp., Chicago, IL) and placed into a Conviron (Winnipeg, MB) growth chamber set on a 28°C/22°C day/night cycle with an 18 hour photoperiod for 3 weeks. Eight jars were used per treatment and contained eight seeds each. Controls included a water soak and a known *F. oxysporum* pathogen. After 3 weeks, the jars were removed from the growth chamber and each seedling was scored as diseased, healthy, or non-emerged. Statistical analyses were performed using Dunnett's one sided test to compare the proportion of healthy seedlings in water control vs. test treatments.

Fusarium isolates were provided by J. Stone and D. Gernandt (Oregon State University). Sixty-one *F. oxysporum* tissue isolates were obtained from Douglas-fir (*Pseudotsuga menziesii* Franco) and Ponderosa pine (*Pinus ponderosa* Laws.) seedlings at Medford (54) and Bend Pine (7) nurseries in Oregon. Thirty-two soil isolates originated at Medford, Oregon (23), Lucky Peak, Idaho (1), Wind River, Washington (3) and Humboldt (2) and Magalia (3) nurseries in California.

RESULTS AND DISCUSSION

Previous work has demonstrated that many *Fusarium* species can be differentiated using PCR and subsequent restriction endonuclease digestions of the histone H3 amplification product (Donaldson et al. 1995; Glass and Donaldson 1995). Five *Fusarium* species have been differentiated by H3-RFLPs. These primers amplify a variety of fungi including representatives of the genera *Botrytis*, *Trichoderma*, *Sclerotinia*, and *Fusarium*, but do not amplify conifer or basidiomycete H3 genes (Glass and Donaldson 1995). A blind test of 45 isolates showed almost 90 percent accuracy, with 39 of 45 tested isolates identified correctly (Donaldson et al. unpublished). Isolates used in the blind test included a variety of fungi often found in conifer nurseries such as *Trichoderma* and *Cylindrocarpon*, as well as several *Fusarium* species which had not been previously examined for H3-haplotype. Thus, PCR amplification of the H3 gene has potential as a tool for detection and identification of *Fusarium* species in environmental samples such as soil and conifer tissue.

In this study, a total of 40 tissue isolates from two nurseries, and 28 soil isolates from five nurseries were examined using H3-RFLPs. Only six H3-haplotypes were observed, providing further evidence that intraspecific variation of the histone H3 gene is not sufficient to interfere with species identification. A Chi-square test indicated no significant difference between soil and tissue populations of *F. oxysporum* at Medford nursery ($P = 0.244$) based on the frequency of H3-haplotypes. Another Chi-square test of available data from Medford nursery indicated no association between H3-haplotype and conifer host among tissue isolates of *F. oxysporum* ($P = 0.419$).

Amplified fragment length polymorphisms were used to characterize 61 tissue isolates from two nurseries and 32 soil isolates from five nurseries. A total of 14 AFLP haplotypes were observed in isolates of *F. oxysporum* from Medford nursery in Oregon; 10 in tissue isolates, and 6 in soil isolates. Only two AFLP haplotypes were present in both tissue and soil isolates. A simple Chi-square test showed a significant difference between soil and tissue isolates of *F. oxysporum* at the Medford site ($P < 0.001$). However, confidence intervals constructed to bind AFLP haplotype frequencies still overlap.

Presumably, additional sampling would resolve the issue of confidence interval overlap. As with the H3-RFLP data, the AFLP data indicate no association between conifer host and AFLP haplotype among tissue isolates of *F. oxysporum* (P = 0.189).

Pathogenicity testing was initiated, and approximately 10 isolates were classified as weakly virulent. Although statistical analysis showed no significant difference between these isolates and the water control (P> 0.05), a low level of disease was typically present. Not enough pathogenicity data were available to determine if any association exists between either H3-RFLP or AFLP haplotypes and pathogenicity or virulence of *F. oxysporum*. Amplified fragment length polymorphism analysis will be used to screen these isolates and an equal number of high-virulence isolates for genetic markers associated with the pathogenic, highly virulent phenotype.

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LITERATURE CITED

Arnheim, N., and H. Erlich. 1992. Polymerase chain reaction strategy. Annual Review of Biochemistry 61:131-156.

Axelrood, P.E., M. Neumann, D. Trotter, R. Radley, G. Shrimpton, and J. Dennis. 1995. Seedborne *Fusarium* on Douglas-fir: pathogenicity and seed stratification method to decrease Fusarium contamination. New Forests 9:35-51.

Donaldson, G.C., L.A. Ball, P.E. Axelrood, and N.L. Glass. 1995. Primer sets developed to amplify conserved genes from filamentous ascomycetes are useful in differentiating Fusarium species associated with conifers. Applied and Environmental Microbiology 61:1331 -1340.

Foster, L.M., K.R. Kozak, M.G. Loftus, J.J. Stevens, & I.K. Ross. 1993. The polymerase chain reaction and its application to filamentous fungi. Mycological Research 97(7):769-781.

Glass, N.L., and G.C. Donaldson. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61:1323-1330.

Henson, J.M., and R. French. 1993. The polymerase chain reaction and plant disease diagnosis. Annual Review of Phytopathology 31:81-109.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: a Laboratory Manual, Second Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Zabeau, M. and P. Vos, 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. European patent application, bulletin 93/13; publication number 0 534 858 A1.

ALTERNATIVES TO METHYL BROMIDE

AND OTHER CHEMICAL SOIL FUMIGATION

ALTERNATIVES TO CHEMICAL FUMIGATION IN BAREROOT FOREST NURSERIES: EFFECTS ON PATHOGEN LEVELS AND SEEDLING DENSITY, MORTALITY AND QUALITY[1]

J.K. Stone[2], D. Hildebrand[3], R.L. James[4], and S.J. Frankel[5]

ABSTRACT

Our objectives were to develop cultural approaches aimed at reducing populations of soil-borne pathogenic fungi in forest nurseries to reduce or replace conventional chemical soil fumigation. One three-year cycle of this study has been completed in seven nurseries in Oregon, California, and Idaho. A second cycle of experiments has been initiated in six nurseries. In the first cycle, non-chemical, cultural methods, including bare fallow, organic soil amendments, seed cover, and time of sowing were compared with methyl bromide (MC-33) or dazomet soil fumigation in reducing preplant pathogen populations. Bare fallow, with or without periodic tilling, was equivalent to chemical fumigation treatments in reducing preplant levels of *Fusarium oxysporum*. Seedling density and mortality varied among several treatments, but at all nurseries combinations of soil amendments and bare fallowing yielded seedling densities and mortalities equivalent or superior to chemically treated plots. Two-year old seedlings were on average larger and had better developed root systems from fumigated plots in some nurseries. In several nurseries, seedlings from non-fumigated bare fallow plots were equivalent in height and root collar diameter to fumigated treatments. Bare fallowing and other management alternatives may help nurseries control seedling disease problems without or with reduced use of soil fumigants.

INTRODUCTION

Many forest tree nurseries use chemical biocides for soil fumigation treatment prior to sowing. Methyl bromide (67%) with chloropicrin (33%) (MC-33) is one of the most commonly used fumigants, and is generally considered the best for controlling soil-borne diseases, insects, weeds, and nematodes and achieving a uniform, vigorous crop of high-quality conifer seedlings. Dazomet is also used, and its granular form is considered more safe to handle than gaseous methyl bromide mixtures (Landis and Campbell 1991). Because methyl bromide has a high potential to deplete stratospheric ozone, production and importation in the United States will be prohibited after January 1, 2001 (Anonymous 1995; World Meteorological Organization 1995). Since 1994, methyl bromide production in the U.S. has been restricted to 1991 levels. Reliance on any chemical biocide to maintain production is inherently risky over the long term. Such chemicals are likely to become restricted in use due to human or environmental health hazards.

In addition to the health hazards of chemical biocides, fumigation drastically disrupts soil biology, destroying beneficial and detrimental organisms alike (Munnecke and Van Gurdy 1979). Among the first microorganisms to recolonize fumigated soil, either from residual survivors in roots and debris, from blowing dust or from soil fragments on equipment, may be opportunistic pathogens (Vaartaja 1979). Populations of some beneficial microorganisms, including those antagonistic to pathogens, develop slowly, while populations of many opportunistic pathogenic fungi can increase rapidly under favorable conditions (Marx et al. 1989; Stone and Hansen 1994). Opportunistic pathogens such as *Fusarium oxysporum* Schlecht. are relatively common in agricultural soils but infrequent or absent in forest soils (Schisler and Linderman 1984). Absence of *Fusarium* in forest soils may be attributable to microbial antagonists in forest soil communities (Schisler and Linderman 1984). Fumigation can result in a need for continued fumigation due to elimination of beneficial microbial antagonists.

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The cultural cycle employed by many bareroot nurseries, especially those in the Pacific Northwest of the U.S., includes a 2-year production of the main seedling crop followed by a season of cover cropping. The cover crop is usually incorporated as a green soil amendment in late summer. This input of rich organic matter can cause sharp increases in populations of soil fungi such as *F. oxysporum*, which have the capability to grow saprobically and survive at high levels in soil over the winter through formation of chlamydospores (Stone and Hansen 1994). High populations of *F. oxysporum* can be associated with severe disease losses (Stone and Hansen 1994). Many nurseries in the Pacific Northwest correct this problem by fumigating soils in late summer or fall after incorporating cover crops.

Although fumigation may eliminate a number of pest problems simultaneously, alternatives can be developed to address the specific needs at individual nurseries based on their histories of pest problems and cultural practices. Long term reductions in dependence on chemical fumigation relies on development of cultural alternatives that favor microbial antagonists of pathogens and promote diverse soil microbial communities.

A technology development project funded by the USDA Forest Service (Forest Health Protection) entitled "Alternatives to Chemical Fumigation in Bareroot Forest Nurseries" concentrated on management of soil-borne seedling diseases. The goal was to enable nurseries to produce comparable seedling crops without or with reduced use of chemical soil fumigation. Specific objectives were to produce seedling crops having seedbed densities and seedling quality equivalent to those grown with conventional fumigation.

MATERIALS AND METHODS

Study sites included five USDA Forest Service nurseries: Coeur d'Alene Nusery in Coeur d'Alene, ID; Lucky Peak Nursery near Boise, ID; Placerville Nursery near Placerville, CA; Bend Pine Nursery in Bend, OR; and J. Herbert Stone Nursery near Medford, OR. Treatments employed varied among nurseries according to individual nursery pest problems and management concerns. Presow soil treatments included cover cropping, fallowing, fumigation, and incorporation of soil amendments. Mulch treatments covered seed after sowing. Chemical fumigants used for comprison were those conventionally used at each nursery. Conifer species tested represented a major crop species produced at each nursery. A single seedlot was sown into all treatment plots within a nursery, except as noted. All study plots were established in spring 1993, prior to soil treatments.

Measurement of seedling density and mortality:

For the first growing season, seedling densities (seedlings/m^2) were recorded approximately three weeks after emergence, at mid-season and at the end of the first growing season (1-0). Three fixed plots 0.15m x 1.22m, oriented across beds were established in each treatment replicate plot for each nursery. Total numbers of live seedlings, seedlings killed by disease (post-emergence damping-off and root disease), and seedlings killed by other causes were recorded for each fixed plot for each measurement period. Cumulative percent mortality from disease during the first growing season beginning with the emergence count and ending with the last first-season count (1-0) was used for statistical analyses. Final density was taken at the end of the second growing season (2-0) for three randomly located plots as described above in each replicate plot.

Measurement of seedling quality:

Seedling shoot length and root collar diameter were recorded for a sample of 50-100 seedlings from each replicate plot in each nursery at the end of both the first and second growing seasons. Measurements were made using the Machine Vision seedling inspection system, a line scanning digitizer image analysis developed by the University of Oklahoma, using the software provided by the manufacturer. Use of the Machine Vision system was kindly provided by the USDA Forest Service J. Herbert Stone Nursery, where it is routinely used for grading seedlings.

Soil sampling:

Soil samples were collected for determination of *Fusarium* spp. and *Pythium* spp. levels at three times for each nursery: spring 1993 at the time plots were established after incorporation of soil amendments, fall 1993 at approximately 3 weeks following fumigation treatments, and in spring 1994 immediately prior to sowing. Soil samples were collected in polyethylene bags, transported to the laboratory in coolers, stored at 4°C, and processed within 48 hours of collection. Soil samples were paseed through a 0.6cm screen and 10g (fresh wt) added to flasks containing 10ml of 0.1% water agar. These samples were mixed and serially diluted (1:10, 1:100) in 0.1% water agar for plating. A portion of each soil sample was oven-dried for determination of water content and conversion of propagule counts to a dry weight basis.

Four plates of each sample, 0.5ml diluted soil on each plate, were prepared on a selective medium. Komada's medium (Komada 1975), modified with an amendment of 1g/l LiCl for suppression of *Trichoderma* spp. (Wildman 1991) was used for enumeration of *F. oxysporum*. Dilution plates of Komada's medium were incubated under fluorescent light and read after 6 days. The average number of colonies on four plates multiplied by the dilution factor and soil water conversion factor yielded colony-forming units per gram of oven-dried soil (CFU).

Statistical analyses:

Statistical analyses were carried out using SYSTAT (SYSTAT 1992) or SAS software (SAS 1992). ANOVA tests with Tukey's or Scheffe's multiple comparison procedures were used for comparisons of seedling quality factors among treatments at each nursery. Seedling density and mortality data were logit transformed (Sabin and Stafford 1990) and analyzed using ANOVA. Mean CFU for each replicate plot were analyzed using the Kruskal-Wallis procedure and the Mann-Whitney U statistic for comparisons between treatments.

Study sites:

Bend Pine Nursery:

The entire study block at this nursery received 200 cu yd/A aged sawdust with 300 lb/A of 34-0-0 nitrogen. Treatments included 3 bare fallow and 2 with pea cover crops sown in May, 1993. One bare fallow treatment had tilling at 3-week intervals; and the other two had no tilling. One bare fallow without tilling treatments had a mulch of pine needles (pine mulch) collected locally the previous fall and applied after sowing to cover seed. The treatment representing standard nursery practice was fumigation with MC-33 in September, 1993, at approximately 392kg/ha, after incorporation of the pea cover crop. The same cover crop treatment without fumigation provided a comparison. Seedbeds were formed and ponderosa pine (*Pinus ponderosa* Laws.) sown in late April, 1994. The study area was a block 122m x 15m, divided into 32 plots. Five replicates of each of five treatments were randomly assigned to plots, leaving seven unused plots.

J. Herbert Stone Nursery:

All treatments included bare fallow, four with tilling at 3-week intervals, and one without tilling. Sawdust was incorporated as a soil amendment in May, 1993 for four treatments. Supplemental nitrogen was included with the sawdust in three treatments (SD+N). In the fourth sawdust treatment, extra nitrogen was provided only after seedling emergence (SD delayed N). The treatment representing standard nursery practice was sawdust with nitrogen, periodic tilling, and fumigation with dazomet in September, 1993, at approximately 392kg/ha. Seedbeds were formed with Douglas-fir (*Pseudotsuga menziesii* Franco) and ponderosa pine sown in late April, 1994. The study area was 213m x 17m, divided into 27 plots. Five replicates of each of 5 treatments were randomly assigned to plots, leaving two unused plots. Each plot consisted of one bed of ponderosa pine and one of Douglas-fir.

Coeur d'Alene Nursery:

All treatments included bare fallow with tilling. Fields were kept weed free by periodic tilling for 1 year prior to sowing. Two soil organic amendment treatments were incorporated in late June, 1993. These were composted bark chips (bark compost) and sewage sludge, a composted mixture of sludge with wood waste (results not discussed in this paper). The "pine mulch" treatment was a layer of pine needles (collected locally the previous fall) applied after sowing to cover seed. The fumigation treatment was dazomet applied at approximately 392kg/ha, water sealed and rolled in late September, 1993. Seedbeds were formed and Douglas-fir (*P. menziesii* var. *glauca* [Beissn.] Franco) sown in early May, 1994. The study area was 122m x 7m, divided into 32 plots. Five replicates of five treatments were randomly assigned to plots, with seven plots unused.

Lucky Peak Nursery:

All treatments were kept bare fallow for 1 year before sowing, four without tilling and one with tilling at 1-month intervals between mid-June and late September 1993. Two soil amendments were incorporated in early June, 1993. These were mushroom compost (m compost), waste composted material used in the commercial production of mushrooms, and commercial sawdust containing supplemental nitrogen (SD+N). The standard nursery fumigation treatment was MC-33 applied at approximately 392kg/ha in September. Two study areas were established in different fields, each 106m x 9m. Each study area was divided into 35 plots; five treatments randomly assigned to the plots, leaving 10 plots unused. One study area was sown with a single seedlot of ponderosa pine and the other with three different seedlings of lodgepole pine (*Pinus contor-*

ta Dougl.). The seedbeds were formed and sown in late May, 1994.

Placerville Nursery:

All treatments began with bare fallow and tilling every few weeks, followed by an over-winter soil covering of rice straw, sawdust, pine needles, dry hydromulch, or nothing (bare soil). Shasta red fir (*Abies magnifica* A. Murr. var. *shastensis* Lemm.) seed was sown in March (early) or mid-April (late), and covered with soil, hydromulch, or sawdust. The nursery had no fumigation treatment. The study site was made up of 4 blocks, each 64m x 3m; each block was divided into seven plots, with seven treatments randomly assigned within each block.

RESULTS

Bend Pine Nursery:

At the Bend Pine Nursery, mean seedling bed density was equivalent among the fumigated and three bare fallow treatments, and mean seedling mortality was not significantly different ($P=0.05$) among these four treatments (Table 1). Methyl bromide fumigation reduced preplant *Fusarium* populations, but not to below detectable limits. Mean presow *Fusarium* levels for the three bare fallow treatments were not statistically different from the fumigated treatment. Presow *Fusarium* populations were much greater in the pea cover crop without fumigation, and seedling mortality was correspondingly much greater (Table 1).

Seedling quality was similarly comparable for the three bare fallow and fumigation treatments; mean 2-0 ponderosa pine seedling shoot height was significantly greater in the fumigation treatment and similar in all other treatments. All treatments resulted in seedlings with mean heights well within the acceptable standards of 10-40cm for 2-0 ponderosa pine (Table 1). Mean root collar diameter measurements for all treatments were also well above the minimum acceptable 4mm. Seedlings from the bare fallow treatments had similar mean calipers; those from the methyl bromide fumigation were significantly larger. Mean caliper of seedlings from pea cover crop without fumigation was much greater, the result of very low bed densities (one-third that of the other treatments), with the same amount of seed and fertilizer as the other treatments. Cost of seed and fertilizer for seedlings grown under this regime would be three times that of seedlings grown under the other treatments.

J. Herbert Stone Nursery:

At J. Herbert Stone Nursery, dazomet fumigation reduced presow *Fusarium* levels, but not to below detectable levels. *Fusarium* populations were significantly greater only in the one treatment without tilling, which had excessive weed growth. The weeds were mainly *Portulaca oleraca* L. that resulted in increased *Fusarium* populations in much the same way as a cover crop. Although mean bed densities for this treatment were lower than other treatments for both ponderosa pine and Douglas-fir, these effects were significant at $P<0.05$ only for Douglas-fir and only between the no till treatment and the tilling with sawdust and delayed N fertilization (Table 2). Mortality was relatively low for this treatment in ponderosa pine (Table 3); however this may reflect the difficulty of detecting seedlings emerging in plots with dense weed cover. Although mean seedling mortality for Douglas-fir was not significantly different among treatments (Table 2), mortality may have been underestimated in the no till treatment due to weed cover.

Ponderosa pine seedlings averaged somewhat smaller for the no till treatment (root collar diameter different at $P<0.06$), but still within acceptable limits for all treatments (Table 3). Mean Douglas-fir seedling height was within acceptable limits for wet site 2-0 Douglas-fir, 20-50cm, except in the no till treatment. Differences among treatments were statistically significant only between the no till treatment and dazomet fumigation and tilling without sawdust treatment. Mean Douglas-fir caliper was well above the acceptable minimum of 4mm for all treatments (Table 2). Smaller, less vigorous seedlings in the no till treatment as compared to other treatments was probably related to the weed infestation and may reflect combined competition with weeds and injurious but non-lethal root colonization by *Fusarium* or other pathogens.

Table 1. Effects of selected soil treatments on ponderosa pine seedling density, morphology, mortality and *Fusarium* populations aat the Bend Pine Nursery, Oregon.[1]

Treatment[2]	Density[2]	Diameter (mm)	Height (mm)	Mortality[4]	Initial Fusarium[5]	Pre-sow Fusarium[5]
Peas, MC-33	226a	6.8b	25.4a	5.5a	31948	170a
Fallow, till	237a	6.2a	19.1b	11.4a	9583	618a
Fallow	226a	6.1a	20.4b	9.3a	2333594	948a
Pine mulch	226a	5.8	15.9b	na	4530	219a
Peas, No fum.	75b	8.5	19.6	39.1b	4088	3711b

[1] Within each column, means followed by the same letter are not significantly different (P=0.05) using Tukey's multiple comparison procedure.
[2] Sawdust with nitrogen was incorporated into all plots at the beginning of treatment.
[3] Number of 2-0 seedlings/m².
[4] Percent of seedlings killed by disease during the first growing season.
[5] CFU per gram dry weight of soil.
[6] Pine needle mulch prevented accurate emergence counts.

Table 2. Effects of selected soil treatments on Douglas-fir seedling density, morphology, mortality and *Fusarium* populations at the J. Herbert Stone Nursery, Medford, OR[1]

Treatment[2]	Density[2]	Diameter (mm)	Height (mm)	Mortality[4]	Initial Fusarium[5]	Pre-sow Fusarium[5]
Till, S+N, Fum.	210ab	7.4ab	29.2a	9.9	1157	135a
Fallow, S+N	182ab	6.5a	21.8ab	13.1	506	2194a
No till, S+N	160a	6.6ab	17.8b	15.7	1193	3469b
Till, no S	206ab	8.0b	29.6a	11.7	415	1106a
Till, S+del.N	256b	6.9ab	27.4ab	9.8	516	808a

[1] Within each column, means followed by the same letter are not significantly different (P=0.05) using Tukey's multiple comparison procedure.
[2] All treatments were bare fallow; fumigation was with dazomet; treatment 5 had delayed application of nitrogen.
[3] Number of 2-0 seedlings/m².
[4] Percent of seedlings killed by disease during the first growing season.
[5] CFU per gram dry weight of soil.

Table 3. Effects of selected soil treatments on ponderosa pine seedling density, morphology, mortality and *Fusarium* populations at the J. Herbert Stone Nursery, Medford, OR[1]

Treatment[2]	Density[3]	Diameter (mm)	Height (mm)	Mortality[4]	Initial Fusarium[5]	Pre-sow Fusarium[5]
Till, SD+N, Fum.	214	8.5	31.5a	9.1	1157	135a
Till, SD+N	230	8.8	31.0a	2.7	506	2194a
No till, SD+N	180	7.8	22.6b	3.3	1193	3469b
Till, no S	225	8.8	31.7a	6.5	415	1106a
Till, S+del.N	250	8.1	29.8a	6.0	516	808a

[1] Within each column, means followed by the same letter are not significantly different (P=0.05) using Tukey's multiple comparison procedure.
[2] All treatments were bare fallow; fumigation was with dazomet; treatment 5 had delayed application of nitrogen.
[3] Number of 2-0 seedlings/m².
[4] Percent of seedlings killed by disease during the first growing season.
[5] CFU per gram dry weight of soil.

Coeur d'Alene Nursery:

At Coeur d'Alene Nursery, dazomet fumigation reduced *Fusarium* populations but not to below detectable levels. Mean presow *Fusarium* levels were significantly greater in the sewage sludge compost treatment than for the fumigation, sawdust compost and no amendment treatments, but not significantly different from the pine mulch treatment. Mean seedbed densities were not significantly different among the different treatments, despite high first-year mortality in all treatments (Table 4).

For dry-site 2-0 Douglas-fir, the minimum acceptable seedling height is 15 cm and 5 mm caliper; average seedling dimensions from all treatments were within these parameters. Mean seedling height differed significantly only between seedlings grown in the dazomet fumigation treatment and sawdust compost treatment. Mean caliper did not differ significantly among treatments (Table 4).

Lucky Peak Nursery:

At Lucky Peak Nursery, methyl bromide fumigation reduced *Fusarium* levels compared to the other treatments, but not to below detectable levels. Both study sites had comparatively low initial *Fusarium*; levels in the mushroom compost treatment increased slightly in the ponderosa pine field, and somewhat more in the lodgepole pine field (Tables 5 and 6). Mean seedling mortality for ponderosa pine was lowest in the methyl bromide fumigated treatment and highest in the bare fallow without tilling or soil amendment, but the only significant differences among treatment means were between fumigated and bare fallow without tilling. Mean seedling mortality in the sawdust plus N amendment was comparable to that for the methyl bromide fumigation. Mean seedling density was lowest for the bare fallow without tilling; however, this difference was significant only at the $P<0.1$ level (Table 5).

Mean ponderosa pine seedling heights were well within acceptable limits of 10-40cm for 2-0 stock for all treatments. Mean heights were similar in the bare fallow alone and fumigation treatments, and these were significantly greater than either soil amendment treatment. Mean caliper was well above the minimum 4mm for all treatments, and showed a similar pattern to height with respect to treatment effects (Table 5). Although seedlings in the bare fallow alone treatment had larger mean caliper and height compared to other treatments, these seedlings also had lower mean bed densities and the greater size may reflect lower densities.

Table 4. Effects of selected soil treatments on Douglas-fir seedling density, morphology, mortality and *Fusarium* populations at the Coeur d'Alene Nursery, ID[1]

Treatment[2]	Density[3]	Diameter (mm)	Height (mm)	Mortality[4]	Initial Fusarium[5]	Pre-sow Fusarium[5]
Dazomet	315	6.0	24.1a	64.0	350	73a
Sawdust comp.	297	5.0	16.1b	61.9	333	217a
Bare fallow	285	6.0	20.0a	53.2	266	172a
Pine mulch	290	6.0	20.0a	na[6]	577	1329ab
Sludge comp.	296	na[7]	na[7]	53.6	679	2180b

[1] Within each column, means followed by the same letter are not significantly different (P=0.05) using Tukey's multiple comparison procedure.
[2] All treatments included bare fallow with tilling; sawdust and sludge organic amendments were composted prior to incorporation.
[3] Number of 2-0 seedlings/m².
[4] Percent of seedlings emerged that were no longer present in plots at the end of the first growing season; not all mortality likely due to disease.
[5] CFU per gram dry weight of soil.
[6] Pine needle mulch prevented accurate emergence counts.
[7] Data not available.

Table 5. Effects of selected soil treatments on ponderosa pine seedling density, morphology, mortality and *Fusarium* populations at the Lucky Peak Nursery, Boise,ID[1]

Treatment[2]	Density[3]	Diameter (mm)	Height (mm)	Mortality[4]	Initial Fusarium[5]	Pre-sow Fusarium[5]
Fallow, till	229	6.5b	25.4b	13.9ab	448	496
Fallow	191	7.0b	27.0b	17.1b	81	241
Fallow, comp.	205	6.0a	20.5a	11.3ab	123	227
Fallow, sawdust	249	6.0b	22.5a	7.9ab	310	214
Fallow, MC-33	229	6.6b	27.1b	3.7a	96	80

[1] Within each column, means followed by the same letter are not significantly different (P=0.05) using Tukey's multiple comparison procedure.
[2] Sawdust with nitrogen was incorporated in all plots at the beginning of treatment.
[3] Number of 2-0 seedlings/m².
[4] Percent of seedlings killed by disease during the first growing season.
[5] CFU per gram dry weight of soil.

Table 6. Effects of selected soil treatments on lodgepole pine seedling density, morphology, mortality and *Fusarium* populations at the Lucky Peak Nursery, Boise,ID[1]

Treatment[2]	Density[3]	Diameter (mm)	Height (mm)	Mortality[4]	Initial Fusarium[5]	Pre-sow Fusarium[5]
Fallow, till	182a	4.7	15.8ab	18.3ab	189	456
Fallow	220a	4.5	16.4ab	16.0ab	468	510
Fallow, comp.	150a	4.4	13.4b	29.7b	28	655
Fallow, sawdust	178a	4.6	15.9ab	21.0ab	151	1020
Fallow, MC-33	217b	4.8	18.9ab	11.0a	84	108

[1] Within each column, means followed by the same letter are not significantly different (P=0.05) using Tukey's multiple comparison procedure.
[2] Sawdust with nitrogen was incorporated in all plots at the beginning of treatment.
[3] Number of 1-0 seedlings/m^2.
[4] Percent of seedlings killed by disease in the first growing season.
[5] CFU per gram dry weight of soil.

Seedling mortality for lodgepole pine was significantly greater in the mushroom compost treatment compared to the fumigation treatment. These two means were significantly different at $P<0.05$. Seedling mortality in the other bare fallow treatments were not statistically different from the fumigation treatment. Mean seedling densities were similar for fumigation and bare fallow alone, and these two treatment means were significantly different from bare fallow with tilling and both bare fallow treatments amended with either sawdust or mushroom compost (Table 6).

Placerville Nursery:

At Placerville Nursery, *Fusarium* levels increased in all treatments between the initial sampling and sowing. Seedling mortality, however, was high only for the treatment with late sowing and soil covering of seed. Seedling densities were correspondingly low for this treatment compared to all others (Table 7). Surviving seedlings from this treatment were more vigorous than seedlings from the other treatments and mean caliper and height were significantly greater than the other treatments (Table 7). All other treatments had similar mean bed densities, caliper, and height. All treatments resulted in mean caliper exceeding the 4 mm minimum and mean heights exceeding the 8cm minimum.

DISCUSSION

Seedling root collar diameter is the most important measure of seedling quality specified by reforestation customers. Seedbed density determines the cost per unit area of growing space. These two measures of seedling quality should, therefore, provide a suitable basis for comparison of seedlings produced under non-fumigation alternatives with standard fumigation. In experiments at nurseries comparing the effects of chemical soil fumigation before sowing with non-fumigation management alternatives, seedling densities and caliper were comparable for fumigated and non-fumigated treatments after two growing seasons. Similar results have been previously reported (Hildebrand et al. 1995; Stone and Hansen 1994). Seedbed density reflects a combination of factors including seed quality, sowing density, first-year losses due to disease, and non-disease mortality. In this study, mortality losses due to post-emergence disease were determined separately, but pre-emergence losses were not determined. Final seedbed densities, therefore, reflect a combination of undetermined pre-emergence mortality, post-emergence mortality, and other incidental losses.

For some nurseries, conventional practices may make use of chemical fumigation necessary. Of the nurseries participating in this study, only Bend Pine Nursery routinely uses inter-rotational cover crops and methyl bromide (MC-33) fumigation. Incorporation of the cover crop as a green soil amendment at the end of the growing season results in increased levels of soil *Fusarium* spp. as well as *Pythium* spp. and leads in turn to high levels of first-year seedling mortality unless corrected by preplant fumigation (Hansen et al., 1990; Stone and Hansen 1994). Our results indicated that bare fallowing may eliminate the need for routine fumigation in some nurseries.

Table 7. Effects of selected soil treatments sowing date, and seed covering on Shasta red fir seedling density, morphology, mortality and *Fusarium* populations at the Placerville Nursery, CA[1]

Treatment[2]	Density[3]	Diameter (mm)	Height (mm)	Mortality[4]	Initial Fusarium[5]	Pre-sow Fusarium[5]
S-BS-late	199a	5.1a	16.5a	41.4a	3386	5285
S-H-early	322b	5.0ab	15.6ab	12.2b	1050	4460
SA-H-early	288b	4.6b	14.4b	14.9b	874	3821
SA-SA-early	299b	4.8ab	15.5ab	14.8b	872	3244
NE-H-early	284b	5.0ab	15.4ab	10.5b	1004	4708
H-H-early	279b	4.9ab	15.7ab	13.1b	1680	5406
BS-H-early	259b	4.6b	14.4b	15.7b	966	3233

[1] Within each column, means followed by the same letter are not significantly different (P=0.05) using Tukey's multiple comparison procedure.

[2] Sawdust and nitrogen was incorporated in all plots at the beginning of treatments. The first letter(s) refer to pre-sowing treatment, the second to seed covering, and the "early" or "late" refer to time of sowing (see text). S=straw; BS=bare soil; H=hydromulch; SA=sawdust; NE=pine needles.

[3] Number of 2-0 seedlings per/m^2.

[4] Percent of seedlings killed by disease during the first growing season.

[5] CFU per gram dry weight of soil.

Populations of soil-borne *F. oxysporum*, a pathogenic fungus responsible for damping-off, hypocotyl rot, and root disease losses of conifer seedlings in the Pacific Northwest (Cordell et al., 1989), are not always reliable indicators of disease potential. Strains of *F. oxysporum* differ in virulence; some strains cause different diseases and certain strains may be nonpathogenic (Bloomberg 1981; Bloomberg and Lock 1972; James 1996; James et al., 1991). Species of *Fusarium* other than *F. oxysporum* can also be responsible for conifer seedling diseases (James et al., 1991; Ocamb and Juzwik 1995). Estimates of *F. oxysporum* in soil were used in this study as a basis for comparing effectiveness of different soil treatments in reducing levels of a soilborne pathogen that is commonly controlled by soil fumigation. Presow levels of soilborne *Fusarium* remained near intitial levels in the bare fallow treatments and were statistically equivalent to levels in fumigated plots. Fumigation was equivalent to bare fallow with or without tilling with respect to seedling bed densities, mortality, and seedling quality at Bend Pine Nursery.

Similar results were obtained in nurseries that do not routinely use cover crops. Dazomet fumiation at the J. Herbert Stone Nursery had no significant beneficial effect on seedling height, caliper, density or mortality for either Douglas-fir or ponderosa pine compared to bare fallow with tilling. The importance of weed control, and the interactions between weed infestation, soil-borne pathogens, and seedling quality were demonstrated in the study at this nursery. High levels of weeds and soil-borne *Fusarium* in one treatment, bare fallow without tilling, were related to significantly higher seedling mortality, reduced bed densities and less vigorous seedlings. Other bare fallow treatments with periodic tilling did not have such serious weed infestation, and seedling densities and quality factors in non fumigated treatments were equivalent to those in the fumigated treatment. Other than a slight difference in seedling height, no beneficial effect from dazomet fumigation was apparent in Douglas-fir seedlings at the Coeur d'Alene Nursery. Similarly, MC-33 fumigation at the Lucky Peak Nursery did not effectively diminish levels of soil-borne *Fusarium* at pre-sow, and seedling densities, mortality, and quality factors were comparable among different bare fallow treatments and fumigation. Placerville Nursery has not been fumigated in recent years because of regulations affecting pesticide use in federal nurseries in the Pacific Southwest Region. Earlier sowing in March and non-soil seed cover reduced first-year mortality compared to the conventional mid- to late April sowing with a soil seed cover.

Nurseries that routinely fumigate prior to planting do so for several reasons. These include control of pests, i.e., pathogenic fungi, weeds, insects, and nematodes, as well as for obtaining vigorous, uniform seedlings. Soil fumigation is a convenient and reliable management tool, since it often effectively eliminates several pest problems simultaneously. The most basic principle of disease managment is to reduce the source of the pest problem, and this often can be achieved by means other than fumigation. Sutherland and Sluggett (1974) found that

numbers of the pathogenic nematode *Xiphinema bakeri* Williams declined in bare fallowed nursery soil, particularly if the soil was frequently cultivated during hot, dry weather. Similar declines in pathogenic fungi were also observed from this treatment that were sufficient to eliminate the need for preplant fumigation in bareroot nurseries in British Columbia (Sutherland, personal communication). Hansen et al. (1990) reported that more packable seedlings were produced in a bare root nursery from a bare fallow treatment than from treatments employing cover crops, whether these were fumigated or not, and despite relatively high incidence of *Fusarium* hypocotyl rot.

Chemical fumigation achieves pest elimination through broad spectrum biocidal activity which removes beneficial as well as harmful organisms. For nurseries that routinely include an inter-rotational fallow year in their production cycle, bare fallowing may be a suitable management alternative for reducing pathogen populations; periodic tilling may further suppress pathogen and weed infestation. Our results suggest that additional fumigation in combination with bare fallow with tilling provides slight, if any, benefit.

Growers who routinely fumigate have fine-tuned watering, fertilizing, and other cultural practices within a fumigated soil system. Seedlings grown under alternative systems may be less uniform than those grown in soil fumigated with methyl bromide/ chloropicrin or other chemical fumigants. Cultural modifications, however, should enable fine tuning of watering, fertilization, and other cultural inputs aimed at improving seeding performance in the absence of fumigation. Modification of cultural practices that can also help reduce or eliminate the need for soil fumigation and other chemical pest control. Our results showed that bare fallowing and other non-fumigation approaches achieved comparable conifer seedling densities, heights, and calipers. Cultural modification such as time of sowing and use of non-soil seed covering can also reduce disease by presenting physical and temporal barriers to infection. Incorporation of slowly decomposing organic soil amendments may encourage increased populations of beneficial microorganisms.

Nurseries have the capability to implement these measures immediately and at reduced cost compared to routine chemical fumigation. Additional disease prevention measures, such as biological control seed treatments can be expected to further reduce disease incidence and severity, in combination with other disease and pest control measures.

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LITERATURE CITED

Anonymous. 1995. Questions and answers on methyl bromide. U.S. Environmental Protection Agency, Office of Air and Radiation, Stratospheric Protection Division, Newsletter. USEPA, Washington, DC. 2p.

Bloomberg, W.J. 1981. Disease caused by *Fusarium* in forest nurseries. *In*: Nelson, P.E., T.A. Toussoun and R.J. Cook (eds.). *Fusarium*: Diseases, Biology, and Taxonomy. The Pennsylvania State University Press, University Park, PA. pp. 178-187.

Bloomberg, W.J. and W. Lock. 1972. Strain differences in *Fusarium oxysporum* causing disease of Douglas-fir seedlings. Phytopathology 62:481-485.

Cordell, C.E., R.L. Anderson, W.H. Hoffard, T.D. Landis, R.S. Smith, Jr. and H.V. Toko. 1989. Forest Nursery Pests. USDA Forest Service, Agriculture Handbook No. 680. 184p.

Hansen, E.M., D.D. Myrold and P.B. Hamm. 1990. Effects of soil fumigation and cover crops on potential pathogens, microbial activity, nitrogen availablility, and seedling quality in conifer nurseries. Phytopathology 80:698-704.

Hildebrand, D., J.K. Stone, R.L. James, S.J. Frankel, J.D. Pokorny, J.G. O'Brien and M.M. Cram. 1996. Alternatives to chemical fumigation technology development project: preliminary results. *In*: Landis, T.D. and B. Cregg (tech. coords.). Proceedings: Forest and Conservation Nursery Associations. USDA Forest Service, General Technical Report, PNW-GTR-365. pp. 15-22.

James, R.L. 1996. Technique for quantifying virulence of *Fusarium* and *Cylindrocarpon* on conifer germinants. USDA Forest Service, Northern Region, Insect and Disease Management. Nursery Disease Notes No. 132. 8p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1991. *Fusarium* diseases of conifer seedlings. *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada, Pacific and Yukon Region. Information Report BC-X-331. pp. 181-190.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review Plant Protection Research (Japan) 8:114-125.

Landis, T.D. and S.J. Campbell. 1991. Soil fumigation in bareroot tree nurseries. *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada, Pacific and Yukon Region. Information Report BC-X-331. pp. 191-205.

Marx, D.H., C.E. Cordell and P. Kormanik. 1989. Mycorrhizae: benefits and practical application in forest tree nurseries. *In*: Cordell, C.E., R.L. Anderson, W.H. Hoffard, T.D. Landis, R.S. Smith, Jr. and H.V. Toko (tech. coords.). Forest Nursery Pests. USDA Forest Service, Agriculture Handbook 680. pp. 18-21.

Munnecke, D.E. and S.D. Van Gundy. 1979. Movement of fumigants in soil, dosage responses and differential effects. Annual Review of Phytopathology 17:405-429.

Ocamb, C.M. and J. Juzwik. 1996. *Fusarium* species associated with rhizosphere soil and diseased roots of eastern white pine seedlings and associated nursery soil. Canadian Journal of Plant Pathology 17:325-330.

Sabin, T.E. and S.G. Stafford. 1990. Assessing the need for transformation of response variables. Oregon State University, College of Forestry, Forest Research Lab, Special Publication 20. 31p.

Schishler, D.A. and R.L. Linderman. 1984. Evidence for the involvement of the soil microbiota in the exclusion of *Fusarium* from coniferous forest soils. Canadian Journal of Microbiology 30:142-150.

Stone, J.K. and E.M. Hansen. 1994. Green manure effects on soilborne pathogens. *In*: Landis, T.D. (tech. coord.). Proceedings of the combined Northeastern and Intermountain Forestry Nursery Associations. USDA Forest Service, General Technical Report. RM-GTR-243. pp. 57-64.

Sutherland, J.R. and L.J. Sluggett. 1974. Time, temperature, and soil moisture effects on *Xiphinema bakeri* nematode survival in fallow soil. Phytopathology 64:507-513.

SAS. 1992. SAS for windows version 3.10. SAS Institute, Inc., Cary, NC.

Systat. 1992. Systat version 5.01. Systat Inc., Evanston, IL.

Vaartaja, O. 1967. Reinfestation of sterilized nursery seedbeds by fungi. Canadian Journal of Microbiology 13:771-776.

Wildman, H. 1991. Lithium chloride as a selective inhibitor of *Trichoderma* species on soil isolation plates. Mycological Research 95:1364-1368.

World Meteorological Association. 1995. Scientific assessment of ozone depletion: 1994, executive summary. Global Ozone Research and Monitoring Project Report No. 37. World Meteorological Organization, Global Ozone Observing System, Geneva, Switzerland. 36p.

USE OF CHROLORPICRIN AS A SOIL FUMIGANT IN PINE NURSERIES[1]

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ABSTRACT

Since 1985, chloropicrin has been tested as a soil fumigant in forest nurseries in Georgia, Mississippi, South Carolina, Texas, Virginia and Wisconsin. The Wisconsin test indicated that when used for reducing soilborne pathogens, chloropicrin (applied at 196 kg/ha with a polyethylene tarp) may be as effective as applying twice as much methyl bromide. Advantages of using chloropicrin as a soil fumigant in southern pine nurseries might include: no plastic tarp required, efficacy similar to methyl bromide with regards to reduction in fungi, nematodes and insects, and it is not a Class 1 ozone depleting substance.

INTRODUCTION

Under authority of the Clean Air Act, the Environmental Protection Agency has assigned a phase-out schedule for methyl bromide with a production termination set for the year 2001. As a result, several organizations are examining alternatives to methyl bromide as a soil fumigant. Some trials involve chemical treatments while others involve non-chemical alternatives. A few of the chemical trials are comparing chloropicrin, dazomet, or 1,3-dichloropropene with methyl bromide. This paper highlights trials with chloropicrin.

On a weight basis, chloropicrin is the second most commonly used fumigant in forest nurseries in the southern United States. In 1994, more than 20,000 kg of chloropicrin were used to produce one billion pine seedlings (South and Zwolinski 1996). Currently, chloropicrin is used in combination with methyl bromide either as a warning agent to enhance safety (2% chloropicrin) or to enhance fungicidal efficacy (33% chloropicrin).

METHODS

Since 1992, the Auburn University Southern Forest Nursery Management Cooperative has conducted soil fumigation trials in Georgia, Mississippi, South Carolina, Texas, and Virginia. Several of the studies included chloropicrin at rates from 140-336 kg/ha. Most of the chloropicrin treatments were applied without using a polyethylene tarp to contain the fumigant. However, a tarp was tested at two nurseries (Statesboro Nursery - Georgia; Summerville Nursery - South Carolina) in 1993 (Table 1).

Basically, studies involved fumigating soil with different chemicals at varying rates and comparing seedling production among the treatments. In 1993 at the Statesboro and Summerville Nurseries, loblolly pine seed were sown in April and May, respectively. At lifting (January, 1994), seedling diameters were measured with a digital caliper. Root-collar diameter (RCD) was used to determine number of cull seedlings (RCD<3.25mm), and the number of grade 2 (RCD 3.25-4.75mm) and grade one (RCD>4.75mm) seedlings. At each nursery, the study involved a randomized complete block design with five replications. Soils at both nurseries were loamy sands. Details of the studies were reported by Carey (1995).

RESULTS AND DISCUSSION

In both the Statesboro or Summerville studies, chloropicrin treatments did not result in significant increases in either total number or in the number of large diameter seedlings produced. For statistical differences to occur, the number of seedlings produced would need to be increased by about 12-14% over other treatments. When compared to not-recently-fumigated soil, chloropicrin (280kg/ha) only increased seedling production by an average of 9% (about 28 seedlings m^2) at the Statesboro Nursery. At the Summerville Nursery, the increase was only 1.5% (about 4 seedlings/m^2). Neither increase was statistically significant at $P=0.05$. However, with a value of $0.04 per seedling, an increase in seedling production of 18/m^2 would be worth about $4,800/ha and a 4/$m^2$ increase would be worth $1,066/ha.

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Table 1. Effects of soil fumigation on 1993 loblolly pine seedlling production at the Statesboro Nursery in Georgia and the Summerville Nursery in South Carolina.

Fumigant	Rate kg/ha	Tarp Used	Number of Seedlings Produced[1] per 0.929m²							
			Statesboro Nursery				Summerville Nursery			
			Total	Ones	Twos	Culls	Total	Ones	Twos	Culls
MC33	392	yes	20.3	7.7	10.8	1.7	29.2	12.9	15.8	0.5
Chloropicrin	280	no	22.1	9.1	11.8	1.1	28.3	9.3	15.5	3.5
Chloropicrin	280	yes	22.6	10.2	9.5	2.8	30.2	9.3	18.8	2.0
Chloropicrin	140	no	19.9	10.1	8.6	1.1	30.7	11.3	16.6	2.7
Chloropicrin	140	yes	21.0	9.0	9.8	2.1	28.6	11.4	14.8	2.3
Dazomet	314	no	20.7	7.3	11.7	1.6	29.0	11.7	16.6	0.7
Dazomet	314	yes	19.2	8.9	8.2	2.1	27.4	13.8	12.5	1.1
Dazomet	157	no	18.6	8.1	8.9	1.6	30.7	9.1	19.8	1.7
Dazomet	157	yes	19.3	7.3	9.1	2.9	30.5	8.3	21.3	0.9
None	0	no	20.8	8.2	10.2	2.3	28.1	6.4	19.6	2.1
None	0	yes	21.5	8.3	10.4	2.7	28.0	6.9	19.3	1.8
Mean - All Treatments			20.6	8.6	9.9	2.0	29.2	10.0	17.3	1.8
LSD			2.59	3.16	3.0	1.88	3.00	5.2	5.7	2.1
P For Treatment Effect			0.08	0.61	0.25	0.49	0.30	0.12	0.11	0.15

[1] Seedlings rated as Ones, Twos and Culls have ground line diameters of greater than 4.76mm, 3.26-4.75mm, less than 3.25mm, respectively.

At a Wisconsin nursery (Table 2), large increases in seedling production resulted from effective soil fumigation with either methyl bromide or chloropicrin (Enebak et al. 1990). Similar increases have also been reported at other nurseries (Sutherland and Adams 1965). It is possible that the absence of large increases in seedling production at southern nurseries might be due to past fumigation practices. For example, low levels of disease currently occurring may be due to improved soil management practices and periodic fumigation in the past which keeps pathogen levels low.

Overall, chloropicrin is a better fungicide than methyl bromide. In some cases, half the amount of chloropicrin was as effective as methyl bromide (Enebak et al. 1990 - Table 2). Chloropicrin is very effective in reducing populations of soil-borne *Fusarium*, *Rhizoctonia* and *Pythium* spp. (Enebak et al. 1990).

Unfortunately, chloropicrin is not as effective as methyl bromide in reducing weed populations. For weed control, it might require 2-1/2 times as much chloropicrin to equal the effectiveness of methyl bromide (Goring 1962). Although chloropicrin does have some herbicidal activity, its use as a fumigant will have to be supplemented with standard herbicides that are effecive in conifer nurseries. If applied appropriately, herbicides are more cost effective for weed control than soil fumigation (South and Gjerstad 1980). However, for control of yellow (*Cyperus esculentus* L.) and purple (*C. rotundus* L.) nutsedge, methyl bromide is still the most effective chemical.

Some nursery managers use economic thresholds (ET) to determine when pesticides should be applied. The ET is the point at which the cost of an option equals the economic impact of the pest controlled (National Research Council 1969). The ET value is determined by the cost of fumigation. Determining if crop loss exceeds the ET value will depend on crop value (Table 3); soil fumigation will be easier to justify when crop value is high. For example, if seed costs $0.06 each and if the manager sows 250 seeds/m², then about $10,000 worth of seed are sown per hectare. The ET of $2,500/ha fumigation would be a 25% reduction in seed loss. It may be surprising to know that the average seed efficiency gain from methyl bromide fumigation (where fumigation had not be done previously) can exceed

Table 2. Effects of soil fumigation on 1986 white pine seedling production at the F.G. Wilson State Nursery in Wisconsin (from Enebak et al. 1990).

Fumigant	Rate kg/ha	Tarp Used	Seedlings Produced Per m^2
Methyl Bromide	392	yes	484
Chloropicrin	196	yes	456
Dazomet	280	no	250
None	0	no	108

25% (Sutherland and Adams 1965). However, today many nurseries sell seedlings that may be 10 times more valuable than seed. At $40/thousand seedlings, the ET for fumigation is reduced to a loss of only 62.5 thousand seedlings. Assuming a production level of 2 million seedlings/ha, this amounts to only a 3% reduction in production. However, the net present value (NPV) of seedlings can be higher than production costs. For some organizations, seedlings that are genetically or morphologically improved have a much higher value than the costs to produce them. For example, the superior performance of large-diameter seedlings, which have a larger root mass, after outplanting compared to small-diameter seedlings is clear. South and Mexal (1984) concluded from numerous published reports that grade 1 seedlings outperformed grade 2 seedlings in both survival and growth. South (1993) concluded that outplanting morphologically improved seedlings (those with 6.5mm diameter instead of 4.5mm) can increase volume production at age 10-20 years by as much as 55 m^3/ha. Using conservative prices, the NPV of grade 1 seedlings can be worth $80/thousand more than grade 2 seedlings. If a nursery produces 100% grade 1 instead of grade 2 seedlings, the ET would be exceeded if production of grade 1 seedlings decreased by 32 thousand (about 1.6%). Therefore, when nursery managers value grade 1 more than grade 2 seedlings, soil fumigation might be relatively easy to justify, even with no increase in number of plantable seedlings.

Table 3. The increase in plantable seedlings required to equal the economic threshold for fumigation ($3000/ha).

Seedling Value	Additional Seedlings Needed per m^2	Additional Seedlings Needed per ha	% Increase in Conifer Stand (2 Million Base)	% Increase in Hardwood Stand (0.5 Million Base)
$0.03	15	100,000	5	20
$0.06	7.5	50,000	2.5	10
$0.12	3.8	25,000	1.25	5
$0.20	2.3	15,000	0.75	3
$0.30	1.5	10,000	0.5	2

When 1+0 seedlings are grown at densities of 200/m^2 in the nursery and are outplanted at 1,000/ha, fumigation of nursery soil with untarped chloropicrin might add $1.12/ha to overall reforestation costs, assuming soil fumigation once every two seedling crops ($2.24/ha assuming soil fumigation once for every seedling crop). We believe this small investment would be good insurance against soilborne pathogens. In some situations, increases in seedling size alone might justify the investment. At a few nurseries, reduction in number of asymptomatic diseased seedlings would justify the cost. For example, the cost of fumigation could be justified if only 7 seedlings/ha died after outplanting due to infection by soilborne nursery pathogens. This mortality amounts to less than 1%. The cost of planting 7 seedlings and treating them with herbicides could exceed $1.12, assuming it costs $0.03/seedling, $0.06 to plant a seedling, and $0.08 to treat with herbicides.

CONCLUSIONS

When production of methyl bromide ceases in the United States, many managers of bare-root nurseries may discover they need to adjust their pest management programs. Some nursery managers in the southern United States may choose to adopt the practice of fumigating with chloropicrin since suppression of pathogens and nematodes with this fumigant is similar to that obtained with methyl bromide. However, some managers may find a need to increase use of selective herbicides, since chloropicrin is not a very effective herbicide.

LITERATURE CITED

Carey, W.A. 1995. Chemical alternatives to methyl bromide. *In*: Landis, T.D. and R.K. Dumroese (tech. coords.). Proceedings: Combined Meeting of the Western Forest Nusery Associations. USDA Forest Service, Rocky Mountain Research Station, General Technical Report RM-GTR-257. pp. 4-11.

Enebak, S.A., M.A. Palmer and R.A. Blanchette. 1990. Managing soilborne pathogens of white pine in a forest nursery. Plant Disease 75:195-198.

Goring, C.A.I. 1962. Theory and principles of soil fumigation. Advances in Pest Control Research 5:47-84.

National Research Council. 1969. Principles of plant and animal pest control.Vol. 3. Insect-Pest Management and Control. National Academy of Sciences. Publication 1695, Washington, D.C. 508p.

South, D.B. 1993. Rationale for growing southern pine seedlings at low seedbed densities. New Forests 7:63-92.

South, D.B. and D.H. Gjerstad. 1980. Nursery weed control with herbicides or fumigation - an economic evaluation. Southern Journal of Applied Forestry 4:40-45.

South, D.B. and J.G. Mexal. 1984. Growing the best seedling for reforestation success. Alabama Agricultural Experiment Station. Forestry Department Series No. 12. 11p.

South, D.B. and J.B. Zwolinski. 1996. Chemicals used in southern forest nurseries. Southern Journal of Applied Forestry 20:127-135.

Sutherland, J.R. and R.E. Adams. 1966. Stand, growth, and nitrogen content of red pine seedlings following chemical treatment of the soil to control disease. Tree Planters' Notes 73:7-10

INTRODUCTION OF INTEGRATED PEST MANAGEMENT IN NURSERIES IN THE CZECH REPUBLIC[1]

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ABSTRACT

Nursery and laboratory experiments were conducted to determine and compare efficacy of several potential biocontrol agents and fungicides and a pre-sowing chilling seed treatment on emergence of Norway spruce (*Picea abies*), European beech (*Fagus sylvatica*), and horbeam (*Carpinus betulus*) seeds. Also, soil assays were made for potential pathogens such as *Fusarium*, *Cylindrocarpon*, *Pythium* and *Phytophthora* before sowing. Four biocontrol agents (*Trichoderma harzianum*, *Pythium oligandrum*, *Bacillus subtilis* and *Azadirachta indica*), the fungicides mancozeb, propamocarb, benomyl and carboxin-thiram applied as seed dressings, a soil drench or granular formulations were tested in two nurseries on spruce and beech and in a greenhouse on hornbeam for their effects on germinant emergence. The chemical or biological materials were applied to wet spruce seeds which were chilled for 3 weeks at 3-5°C. Application of all biological and propamocarb was done on beechnuts before stratification or immediately before sowing. Hornbeam seeds were dressed with both *T. harzianum* and mancozeb before stratification with or without a sand/peat medium. The Norway spruce experiments showed that the biological control agents were as effective as fungicide applications. Occurrence of soil-borne *Fusarium* and *Cylindrocarpon* spp. was less than 500 cfu/g dry soil; *Pythium* spp. were detected at less than 25 cfu/g and no *Phytophthora* spp. were isolated from soil. Best results in nursery plots were observed where granular *A. indica* and *T. harzianum* were applied at the time of sowing. However, emergence speed of spruce germinants was reduced by very cold, wet weather and high sowing densities. Greatest emergence of European beech occurred in plots where beechnuts were treated with *P. oligandrum* or propamocarb both before stratification or sowing. There were no significant differences in emergence of hornbeam seeds stratified with or without sand/peat. However, significantly fewer seedlings were observed in plots with mancozeb-treated seeds. Also, prolonged cold stratification increased speed of hornbeam seed germination.

INTRODUCTION

Integrated pest management (IPM) practices are being developed to reduce pesticide usage and environmental contamination in forest nurseries in the Czech Republic. In 1992 and 1993, laboratory and nursery experiments were conducted to compare efficacy of three potential biocontrol agents (*Trichoderma harzianum* Rifai, *Pythium oligandrum* Drechsler, and *Bacillus subtilis* (Ehrenberg)Cohn) and several fungicides (fenylmercurichloride, Ferrax - a mixture of ethirimol, flutriafol, thiobendazol and imazalil, mancozeb, oxine-sulphate and thiobendazol) against damping-off of Norway spruce (*Picea abies* (L.) Karsten), Scots pine (*Pinus sylvestris* L.) and European larch (*Larix decidua* Miller). Laboratory studies determined *in vitro* effects of the treatments on germination capacity and speed and incidence of seed-borne fungi. The nursery experiments also included assays of soil for seed-borne pathogens. Results of the 1992 nursery experiments suggested that biological control agents could be effective in reducing dependance on fungicides for damping-off control. However, in 1993, the biocontrol fungus *T. harzianum* provided little or no protection against damping-off in the nursery for any of the three tree species tested. Results were more encouraging for *B. subtilis* which was applied as a soil drench, especially for Scots pine. In contrast, neither *B. subtilis* nor *P. oligandrum* provided any protection against damping-off of European larch. The 1993 nursery experiment also showed that certain fungicides, such as oxine-sulphate, which resulted in dramatic increases in numbers of Scots pine seedlings, but not Norway spruce or European larch, may warrant further testing for damping-off control, especially while biological or cultural practices are being perfected (Prochazkova and Nesrsta 1994).

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In 1994, a nursery experiment was conducted using *T. harzianum*, *B. subtilis*, *P. oligandrum*, oxine-sulphate and mancozeb applied to seeds with or without a pre-sowing chilling treatment to determine and compare their efficacy on field emergence of Norway spruce. Also, the occurrence of potential soil-borne pathogens such as *Fusarium*, *Cylindrocarpon*, *Pythium* and *Phytophthora* were determined before sowing. Results showed that there were significantly more Norway spruce seedlings in plots with *B. subtilis*-treated, chilled seeds compared to untreated dry seeds (control), and dry seeds treated with either mancozeb, *T. harzianum*, or *B. subtilis*. Field emergence of chilled seeds was slightly faster but, because of very high summer temperatures and insufficient irrigation, the stimulative effect of pre-sowing treatment later disappeared, and 6 months after sowing there were no significant differences among treatments (Prochazkova et al. in press). Results of previous experiments suggested the possible use of biological control agents if soil and seeds are free of pathogens at the time of sowing. Biological control agents are thought to colonize soil and suppress later pathogen development and spread (Chet and Henis 1983; Munnecke et al. 1981). Together with pre-sowing treatment, they can reduce or minimize use of fungicide or sterilant chemicals (Prochazkova et al. in press).

In 1995 we continued Norway spruce experiments and for the first time conducted experiments with biological control agents applied to dormant seeds of European beech (*Fagus sylvatica* L.) and hornbeam (*Carpinus betulus* L.). We describe results of the 1995 experiments in this paper.

MATERIALS AND METHODS

Seed characteristics and pre-sowing treatments:

Norway spruce seed (provenance Jaromerice, Czech Republic, germination capacity 89%) were placed in thick polyethylene bags with cold water and the suspension or solution of biological or chemical preparations applied as a seed dressing. Seeds were then placed at 3°C for 24 hours after which the water or suspension was drained off and wet seeds were kept at 3-5°C for 3 weeks. Bags were not tightly closed to allow aeration and once a week they were opened and the seeds were mixed. Germination capacity and viability for all three tree species were evaluated according to ISTA International Rules for Seed Testing (1993).

European beechnuts (provenance Romania, viability 74%) were divided into two parts with half the seeds being dressed with either biological or chemical agents before stratification and the second half treated just before sowing. For breaking dormancy, seed was placed in thick polyethylene bags and soaked in cold water or a suspension of the biocontrol or chemical preparations at 3°C for 24 hours. Excess water was then drained off and wet seeds kept in plastic boxes with holes on the bottom for aeration at 3-5°C for 12 weeks.

Hornbeam seed (provenance 1-Traunstein, viability 77%; provenance 2-Furstenbuk, viability 52%; provenance 3-Duffelbeck, viability 75%) were from the 1993 crop from Germany because for 1994-95 there were no fresh or stored hornbeam seed available in the Czech Republic. Before stratification, hornbeam seed were soaked in cold water at 3°C for 24 hours. They were then divided into two parts and one half of each provenance was stratified either with of without sand/peat medium (1:1 w/w). Initially, seeds were kept at 19-21°C for 4 weeks and then at 3-5°C for 16 and 18 weeks. One third of the seeds of each provenance were stratified with or without medium and dressed with either biological, chemical or no agent at the beginning of stratification.

Biological control agents and fungicides:

The biological control agents tested, their trade names in parentheses, manufacturer, application rates and mode of application were: (1) *Trichoderma harzianum*, minimal concentration of 1.4×10^{10}g spores with a minimum germination of 80% (Supresivit®), Fytovita Ltd., Prague, Czech Republic, at 2g per kg of seeds applied on Norway spruce as a seed dressing prior to stratification or as a soil drench at 1g per m² or as a granular formulation at 1g or 2g per m² applied at sowing (Table 1); at 2g per kg on beechnuts as a seed dressing either before stratification or immediately prior to sowing as a granular formulation at 1g per m² applied at sowing (Table 2); at 2g per kg on hornbeam seeds as a dressing before stratification (Table 3); (2) *Pythium oligandrum*, minimal concentration of 1×10^{7}g oospores with a germination of 70% (Polyversum®), Remesla Ltd., Prague, Czech Republic, at 5g per kg of Norway spruce seeds as a seed dressing prior to stratification (Table 1) and of European beech as a seed dressing either prior to stratification or prior to sowing (Table 2); (3) *Bacillus subtilis* (Ibefungin®), ZD "Strachotin", Strachotin, Czech Republic, at 1ml per 1kg of seeds of Norway spruce prior to stratification (Table 1); (4) *Azadirachta indica* (Neem®), at 5g per m² applied in granular form into soil together with seeds at sowing.

The fungicides tested, their trade names in parentheses, manufacturer, and their application rates were: (1) mancozeb 80% (Novozir® MN80), Duslo, s.;. Sala, Republic of Slovakia at 5g per kg of seeds; (2) benomyl (Fundazol® 50 WP) Chinoin, Hungaria at 1g per kg of seeds; (3) carboxin-thiram (Vitavax® 200FF), Uniroyal Chemical Ltd., Broomsgrove, Great Britain, at 2g per kg of seeds; (4) propamocarb (Previcur®SL 607), Hoechst Schering AgrEvo GmbH, Berlin, Germany at 1.5g per kg of seeds. Mancozeb was applied as a seed dressing prior to stratification of hornbeam seeds (Table 3), benomyl and carboxin-thiram were applied to Norway spruce seeds before chilling (Table 1), and propamocarb on beechnuts either before stratification or before sowing (Table 2).

Soil pathogen assays:

When seeds were sown, soil samples were collected for assays of potential pathogens. Komada's medium (Komada 1975) and Nash and Snyder's medium (Nash and Snyder 1962) were used to determine quantitative occurrence of *Fusarium* and *Cylindrocarpon* spp. *Phytophthora* and *Pythium* populations were determined using two selective media for water molds (J. Dennis, personal communication; Hansen et al. 1990). Soil-dilution plates were incubated at 20-22°C in daylight for *Fusarium* and *Cylindrocarpon* and in the dark for *Pythium* and *Phytophthora*. Colonies of *Pythium* and *Phytophthora* were counted after 2 days and *Fusarium* and *Cylindrocarpon* after 7-14 days. Numbers of CFU (colony-forming units) were calculated.

Table 1. Description of Norway spruce treatments for the 1995 experiments.

Treatment	Biological Agents Application Rate	Fungicides Application Rate	Mode of Application
Control	None	None	Chilling[1]
T. harzianum-dressing	2g[2]	None	Dressing before chilling
T. harzianum-soil drench	1g[3]	None	Soil drench at sowing
T. harzianum-granules 1	1g[3]	None	Granules applied into soil at sowing
T. harzianum-granules 2	1g[3]	None	Granules onto soil surface at sowing
T. harzianum-granules 3	2g[2]	None	Granules applied into soil at sowing
T. harzianum-carboxin-thiram	2g[2]	2g[2]	Dressing before chilling
T. harzianum-A. indica	2g[2]+ 5g[3]	None	Seed dressing and *A. indica* granules at sowing
A. indica	5g[3]	None	Granules applied into soil at sowing
B. subtilis	1ml[2]	None	Dressing before chilling
P. oligandrum	5g[2]	None	Dressing before chilling
benomyl	None	1g[2]	Dressing before chilling

[1] Chilling equals stratification without use of sand/peat medium.
[2] Per 1kg of seeds.
[3] Per m² of soil.

Table 2. Description of the European beech treatments for the 1995 experiments.

	Application Rates		
Treatment	Biological Agents	Fungicides	Mode of Application
Control	None	None	Chilling[1]
T. harzianum-before strat.	2g/kg	None	Dressing before chilling
T. harzianum-before sow.	2g/kg	None	Dressing before sowing
T. harzianum-granular.	1g/m soil	None	Granules at sowing with seeds
P. oligandrum-before strat.	5g/kg	None	Dressing before chilling
P. oligandrum-before sow.	5g/kg	None	Dressing before chilling
Propamocarb-before strat.	None	1.5g/kg	Dressing before chilling
Propamocarb-before sow.	None	1.5g/kg	Dressing before chilling

[1] Chilling equals stratification with sand/peat medium.

Table 3. Description of hornbeam treatments for the 1995 experiments.

	Application Rates		
Treatment	Biological Agents	Fungicides	Mode of Application
Control	None	None	Chilling[1]
Control with sand/peat	None	None	Stratification with sand/peat
Control w/o sand/peat	None	None	Stratification without sand/ peat
T. harzianum with sand/peat	2g/kg	None	Stratification with sand/peat
T. harzianum w/p sand/peat	2g/kg	None	Stratification without sand/ peat
Mancozeb with sand/peat	None	5g/kg	Stratification with sand/peat
Mancozeb w/o sand/peat	None	5g/kg	Stratification without sand/ peat

Seed sowing, experimental design, data collection and analysis:

Norway spruce seeds were sown on May 25, 1995 at the Budisov nursery. A completely randomized block experimental design with four replicates of five, 50cm long drill rows was used for each treatment. Healthy seedlings were counted 5, 11, 16 and 26 weeks after sowing. On the first three counting dates, diseased seedlings in each treatment were collected for isolation of pathogenic fungi on selective media.

European beechnuts were sown on May 17 at the Budisov nursery using a completely randomized block experimental design with four replicates of seven, 2m long drill rows for each treatment. Beechnuts were also sown at the Uherske Hradiste Research Station on May 22 using four replicates of 100 seeds per treatment sown in a completely randomized block design. Emerged seedlings were counted 4, 8, 13 and 23 weeks after sowing at the Budisov nursery and 2, 3, 4 and 24 weeks after sowing at the research station.

After 20 weeks of pre-sowing treatment, five replicates of 100 hornbeam seeds for each treatment were sown into a sand/peat mixture (1:1 w/w) in individual boxes in a greenhouse on May 3. After 22 weeks stratification, four replicates of 100 hornbeam seeds were sown on May 16 in seedbeds at the Uherske Hradiste Research Station. Complete randomized block designs were used for both sowings and emergence was evaluated each week for 8 weeks after sowing.

Emergence data were evaluated for each crop species using an analysis of variance. Treatment differences were located using Tukey's multiple range test.

RESULTS

Norway spruce:

Very low numbers of *Fusarium* and *Cylindrocarpon* propagules were isolated from soil samples, e.g. less than 500 cfu/g dry soil. *Fusarium* species (*F. equiseti* (Corda)Sacc. and *F. culmorum* (W.G. Smith)Sacc.) were infrequently isolated from post-emergence damped-off seedlings collected after sowing in all treatments. *Pythium* spp. were detected only from soil at less than 25 CFU/g dry soil and no *Phytophthora* was isolated from either soil or diseased seedlings. Several *Trichoderma* spp. were often isolated from soil samples. Greatest biocontrol was observed in a plot with seeds dressed with *T. harzianum* and granules of *A. indica* applied at the time of sowing (Figure 1). Only treatments with *A. indica*, either alone or in combination with *T. harzianum*, resulted in seedling emergence and survival significantly greater ($P=0.05$) than controls. The least effective treatment was the plot where *T. harzianum* was drenched into soil at sowing (Figure 1). Emergence of seeds dressed with *P. oligandrum* was very good 5 weeks after sowing but, because of severe damping-off, the number of seedlings decreased thereafter. Treatments with benomyl, *B. subtilis* and *T. harzianum* with carboxin-thiram were ineffective in improving seedling emergence (Figure 1). This experiment was affected by very cold, rainy weather and high sowing densities which adversely influenced seedling emergence. For example, 26 weeks after sowing, the greatest decline in seedling numbers occurred in the control plots compared to counts at 5 weeks (decreases of 62%). On the other hand, treatments with applied granules of *T. harzianum* as a drench showed only 2-7% fewer seedlings by the end of the experiment.

European beech:

In Budisov nursery, treatments with *T. harzianum* in granular formulations and with beechnuts dressed with *P. oligandrum* before stratification resulted in significantly ($P=0.05$) less seedlings established (Figure 2). All other treatments were similar to controls and did not significantly improve seedling emergence. There were significantly ($P=0.05$) more seedlings in plots with *P. oligandrum* and propamocarb, both applied just before sowing, compared to *P. oligandrum* and *T. harzianum* applied before stratification. In the trial at the Uherske Hradiste Research Station, no significant treatment effects on seedling emergence were found (Figure 3). However, the speed of germination of beechnuts dressed with *T. harzianum* was signficantly slower than those treated with *P. oligandrum* before sowing. Both these treatments resulted in slower seed germination than beechnuts treated with propamocarb (Figure 3).

Hornbeam:

Stratification with and without sand/peat medium did not signficantly ($P=0.05$) affect seedling emergence for any of the three provenances tested (Figures 4-6). Significantly fewer seedlings emerged in plots with mancozeb-treated seeds of the provenance with highest seed viability (Provenance 1 - Traustein; viability 77%). Number of emerged seedlings was the same for both stratification periods, but the speed of germination was much higher

after prolonged stratification for 22 weeks (Figures 4-6).

CONCLUSIONS

Results of our 1995 nursery experiments showed that, at least for Norway spruce, some biological control agents may be effective in improving seed germination and seedling establishment, thus reducing dependence on fungicides. Results for European beech and hornbeam were less conclusive. However, all experiments were adversely affected by unusual prolonged periods of cool, wet weather during periods of seedling establishment. This undoubtedly affected our results. Therefore, further tests may result in greater effects of biocontrol agents in some crop species. We also showed in these experiments that proper pre-sowing treatments speeds up seed germination and can benefit seedling emergence and quality. Our results showed that if soil and seed are infested with either none or low populations of potential pathogens, it is possible to effectively use biological control agents. These biocontrol agents colonize root rhizospheres and prevent pathogens from either infecting roots or initiating disease. Biocontrol agents used in conjunction with proper pre-sowing treatments can improve seedling establishment and quality and reduce dependence of chemical fungicides.

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LITERATURE CITED

Chet, I. and Y. Henis. 1983. *Trichoderma* as biocontrol agents against soilborne root pathogens. *In*: Parker, C.A., A.D. Rovira, K.J. Moore, P.T.W. Wang and J.F. Kollmorgen (eds.). Ecology and Management of Soilborne Plant Pathogens. Proceedings of Section 5 of the Fourth International Congress of Plant Pathology. pp. 110-112.

Hansen, E.M., D.D. Myrold and P.B. Hamm. 1990. Effects of soil fumigation and cover crop on potential pathogens, microbial activity, nitrogen availability, and seedling quality in conifer nurseries. Phytopathology 80:698-704.

International Seed Testing Association. 1993. International rules for seed testing. Seed Science and Technology 21, Supplement. 277p.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review Plant Protection Research (Japan) 8:114-125.

Munnecke, D.E., M.J. Kolbezen, W.D. Wibur and H.F. Ohr. 1981. Interactions involved in controlling *Armillaria mellea*. Plant Disease 65:384-389.

Nash, S.M. and W.C. Snyder. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soil. Phytopathology 52:567-572.

Prochazkova, Z. and M. Nesrsta. 1994. Experiments using biological controlagents and fungicides against damping-off in bareroot nurseries in the Czech Republic. *In*: Perrin, R. and J.R. Sutherland (eds.). Diseases and Insects in Forest Nurseries. Dijon (France), October 3-10, 1993. Institut National De La Recherche Agronomique. Les Colloques No. 68. pp. 197-211.

Prochazkova, Z., M. Nesrsta and A. Sikorova. 1995. Effect of pre-sowing treatment, fungicides and biological control agents on emergence of Norway spruce (Picea abies(L.)Karsten) seeds. *In*: Proceedings of the workshop: "Forest Seed Collection, Treatment and Storage." Opocno, Czech Republic, June 4-8, 1995 (In press).

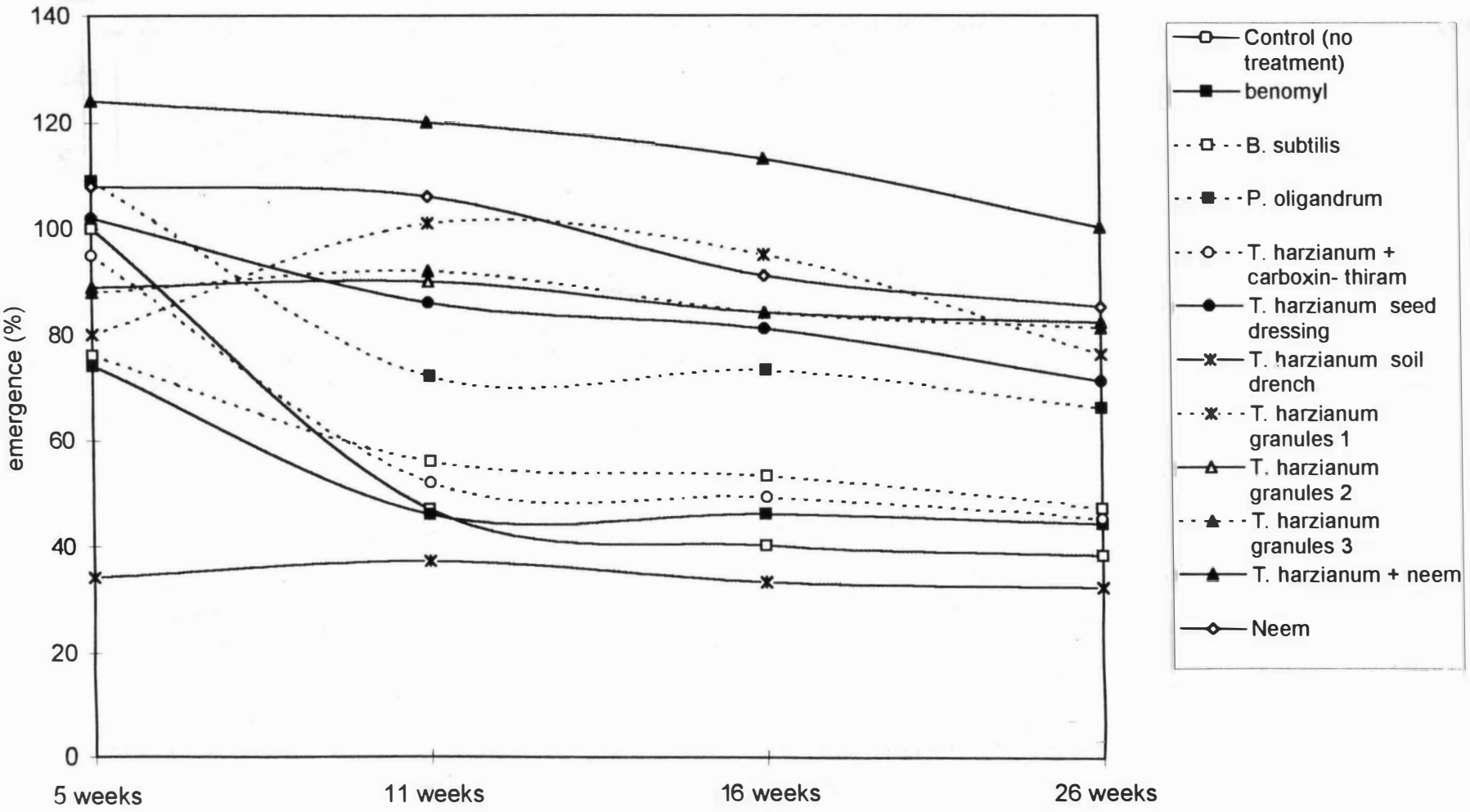

Figure 1. Nursery emergence of Norway spruce seedlings treated with biological control agents and fungicides in 1995.

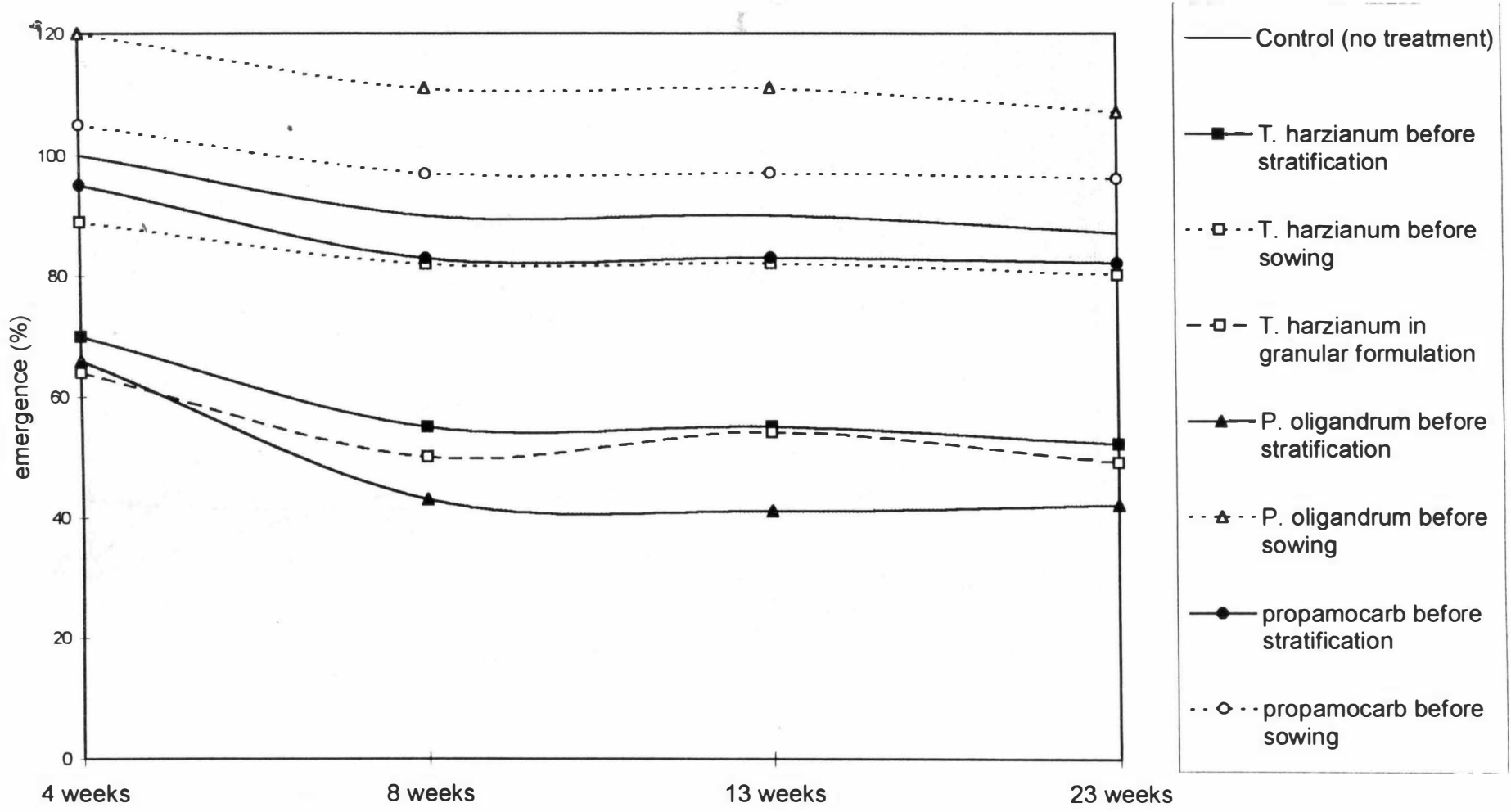

Figure 2. Nursery emergence of European beech seedlings following beechnut treatment with biological control agents and fungicides in 1995 at Budisov.

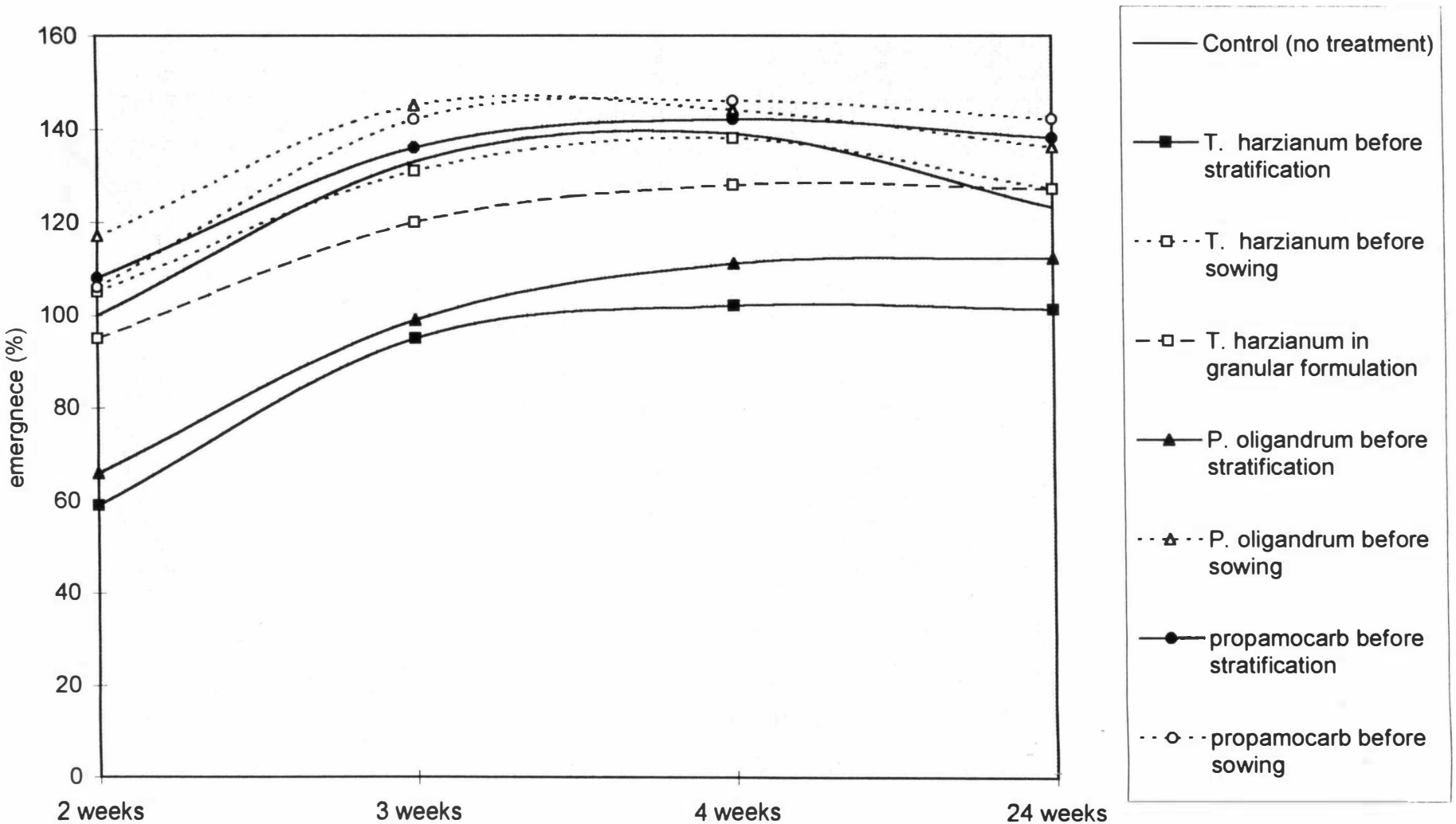

Figure 3. Nursery emergence of European beech seedlings following beechnut treatment with biological control agents and fungicides in 1995 at Research Station Uherske Hradiste.

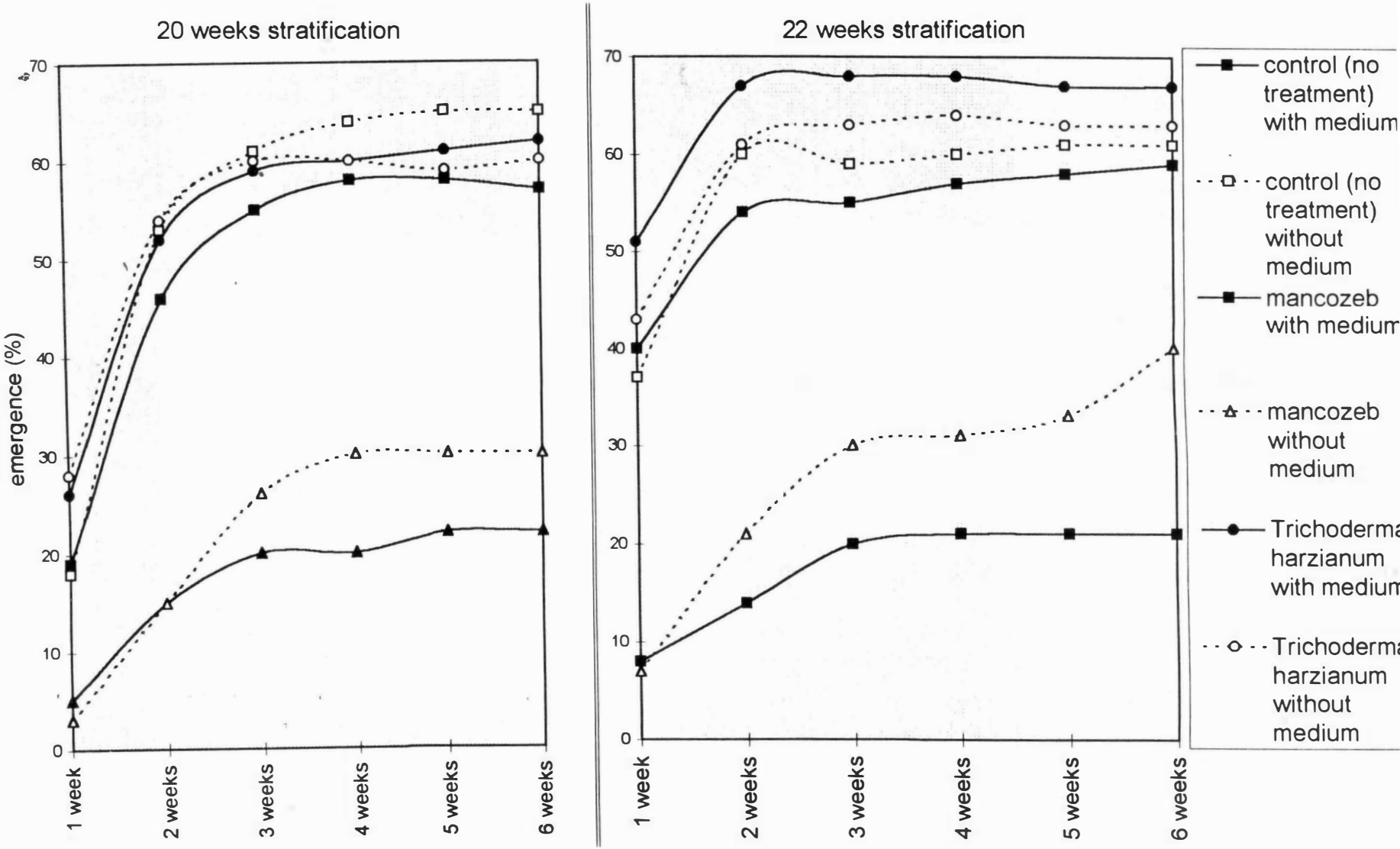

Figure 4. Emergence of hornbeam seedlings following seed stratification with and without medium and treatment with *Trichoderma harzianum* and propamocarb: provenance 1-Traustein, stratification 20 and 22 weeks.

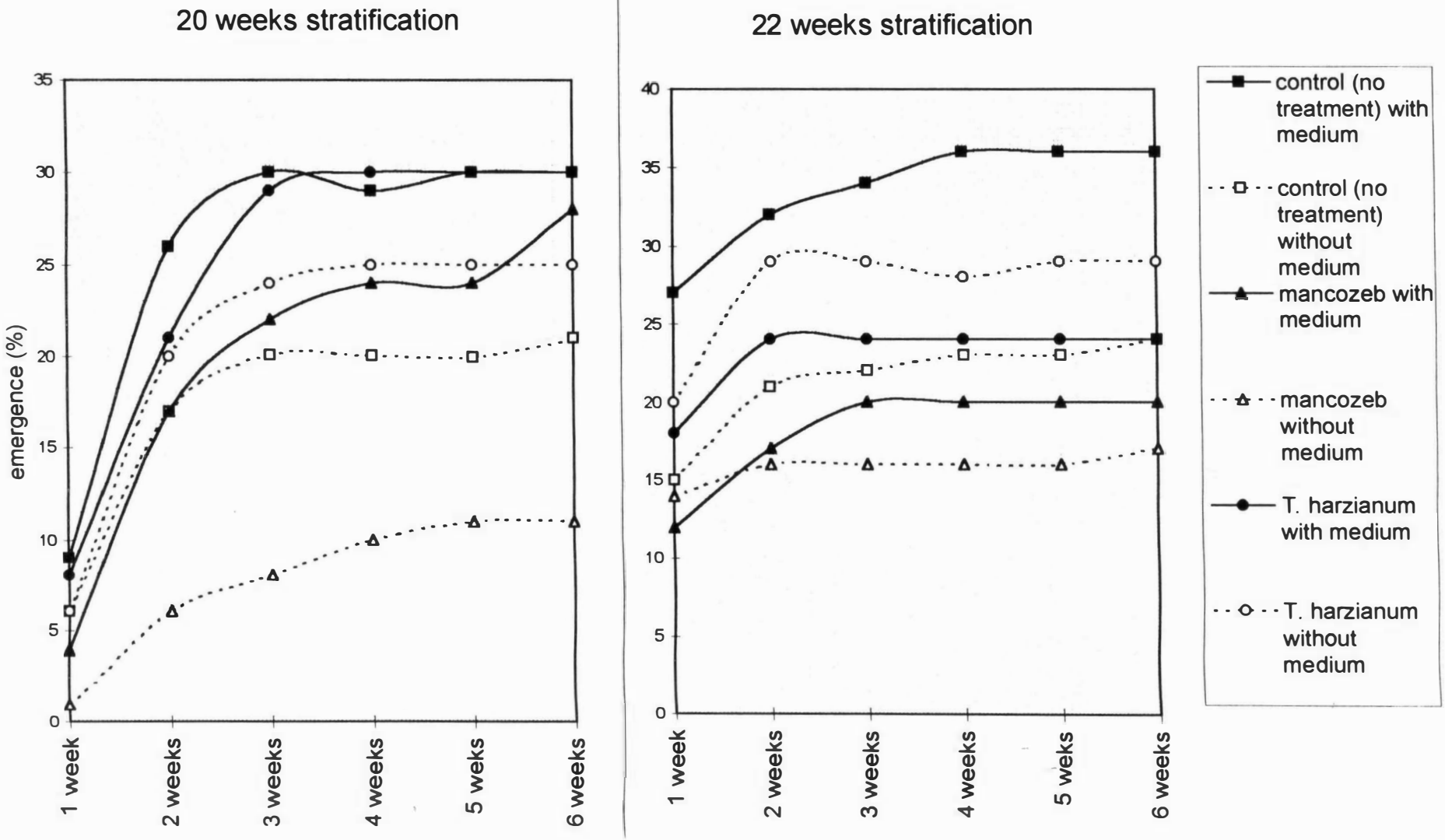

Figure 6. Emergence of hornbeam seedlings following seed stratification with and without medium and treatment with *Trichoderma harzianum* and propamocarb: provenance 3-Duffelbeck, stratification 20 and 22 weeks.

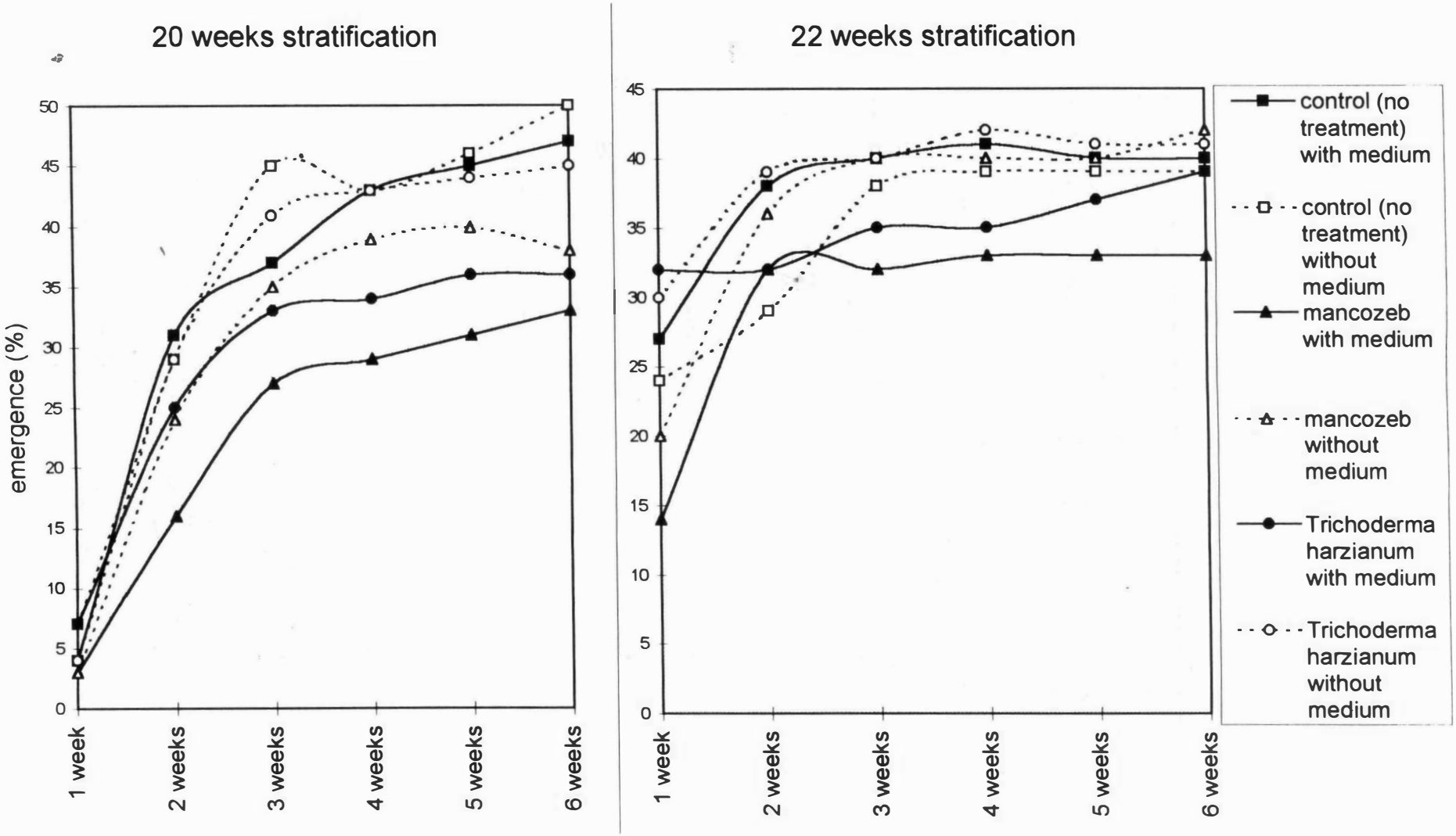

Figure 5. Emergence of hornbeam seedlings following seed stratification with and without medium and treatment with *Trichoderma harzianum* and propamocarb: provenance 2-Furstenbuk, stratification 20 and 22 weeks.

DEVELOPMENT AND FIELD PERFORMANCE OF SLASH AND LOBLOLLY PINE SEEDLINGS PRODUCED IN FUMIGATED NURSERY SEEDBEDS AND SEEDBEDS AMENDED WITH ORGANIC RESIDUES[1]

E.L. Barnard[2], M.E. Kannwishcer-Mitchell[2], D.J. Mitchell[3]
and
S.W. Fraedrich[4]

ABSTRACT

Evaluations of pine seedling production, quality, and disease in southern forest nurseries in soils fumigated with methyl bromide or amended with pine bark or composted organic residues are ongoing. This paper provides a summary of seedling production and quality data for three slash pine and two loblolly pine seedling crops produced in two different southern pine nurseries. In addition, survival and growth data are provided for two of the slash pine seedling crops and one of the loblolly pine seed crops 1 year after seedlings were outplanted operationally on commercial forest sites. To date, treatment effects have been minimal, and statistically significant treatment differences vary among crop-years, treatments, and specific measurement parameters. Evaluation of relative disease suppression efficacies for the soil amendments has been hampered by a general lack of soil-borne pathogen populations and associated root disease development.

INTRODUCTION

The Montreal Protocol assessment of 1991 identified methyl bromide as a chemical contributing to depletion of the stratospheric ozone layer and the U. S. Environmental Protection Agency (EPA) proposed to eliminate production and use of methyl bromide pusuant to the Clean Air Act of 1990 (Civerolo et al. 1993; Smith and Fraedrich 1993). This action has generated a flurry of activity to identify and assess alternatives to methyl bromide for control of soil-borne pests in forest tree nurseries. As part of a national initiative funded by the USDA Forest Service, Forest Health Protection technology development (James et al. 1993), we are comparing effects of pine bark and composted organic residues with methyl bromide soil fumigation on production of southern pine seedlings. The project objectives include (1) assessing effects of organic soil amendments on disease suppression and seedling quality and production, (2) evaluating field outplant performance of treated seedlings, (3) assessing comparative costs and benefits of treatments, and (4) developing methods and baseline data for nursery disease forecasting and risk assessment. Several progress reports have previously been published (Barnard et al. 1994, 1996; Kannwisher-Mitchell et al. 1994). This paper updates results before the fourth and final year of the project. The focus of this paper is on seedling development and field performance. Microbiological and nutrient data for seedlings will be published elsewhere.

MATERIALS AND METHODS

Three nurseries were initially involved in this project. However, unanticipated problems at the USDA Forest Service Ashe Nursery in Brooklyn, MS, resulted in major infestations of nutsedge (*Cyperus* spp.), unusual seed infestations by *Fusarium subglutinans* (Wollenweb. & Reinking) Nelson, Toussoun & Marasas, and mid-study plot relocations. Therefore, we will only report results from our studies of slash pine (*Pinus elliottii* Engelm.) at the Florida Division of Forestry's Andrews Nursery (Chiefland, FL), and loblolly pine (*P. taeda* L.) at International Paper (IP) Company's Supertree Nursery in Blenheim, SC.

Field trials began at the Andrews Nursery in 1993 and the IP Supertree Nursery in 1994. Fumigated study plots received standard operational treatments with methyl bromide and chloropicrin prior to sowing each seedling crop. Plots amended with

[1] Paper presented at the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). Orlando-Gainesville, FL., USA. May 19-24, 1996.

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[4] Forestry Sciences Laboratory, USDA Forest Service, Southern Research Station, Athens, GA, USA.

organic residues received annual applications of either pine bark or composted organic materials. Organic residues consisted of composted yard waste at Andrews Nursery in 1993-94, aged hardwood bark at IP's Supertree Nursery in 1994, and a commercially available composted municipal waste from Tennessee (Bedminster, Inc.) at both nurseries in 1995-96. These materials were applied either at 2.5cm (1X) or 5.0cm (2X) depths and mechanically incorporated into soils to a depth of 15-20cm prior to sowing. Check plots received no treatment other than routine soil tillage and seedbed preparation, which was standard across all treatments. All plots were operationally irrigated, fertilized, and treated with topically applied herbicides. No supplemental treatments were applied to any plots other than the 1996 compost plots at Andrews Nursery. These received an additional 560kg per ha of sulfur to ameliorate treatment-induced pH problems. Plot layout and distribution are described in Figures 1 and 2.

Seedling stand counts were performed periodically (weekly for 4-6 weeks; mid-summer, and end-of-season) within three permanent subplots (0.3 x 1.2m) in the center of each treatment plot. Seedlings from each treatment plot were also systematically sampled at early, mid and end of growing season for comparative morphology measurements, nutrient analyses, and rhizosphere microbe assays. Soil samples collected simultaneously with seedling samples were subjected to standard nutrient and nematological assays. Bacterial, actinomycete, and general fungal populations were assayed from soil from the Andrews' Nursery plots at the beginning, middle, and end of the growing season. In addition, populations of *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., *Phytophthora* spp., and *Macrophomina phaseolina* (Tassi) Goid. were assayed using selective isolation techniques and media (Singleton et al. 1992).

At the end of each crop year, seedlings from each treatment plot were outplanted onto operationally prepared reforestation sites in three replicate 50-seedling row plots in a complete randomized block design. Survival and growth of these seedlings were periodically monitored and measurements taken at the end of the first growing season following outplanting.

RESULTS AND DISCUSSION

Treatment effects thus far have not been large. Although interesting and sometimes subtle treatment differences with respect to rhizosphere microorganisms, seedling nutrition, and seedling size, are apparent, few statistically significant differences were consistent among treatments across study years. Organic residue amendments have clearly influenced soil organic matter and pH. For example, soil organic matter in composted yard waste-amended soils (2X rate) in the Andrews Nursery was above 2.0% after two seedling crops, while that in all other soils was between 0.5-1.0%. Similarly, seedbed soil pH in the Andrews Nursery was well above 6.5 after 2 years of amending with composted yard waste, whereas pH values in all other treatments were approximately 5.0. However, overall differences in seedling quality and field performance have been minimal. Tables 1-4 provide a summary of our seedling quality and performance data to date. Field performance data for the 1995 seedling crops will be collected during the winter of 1996-97, and 1996 crop data are still being collected.

Serious root disease problems did not occur in our study plots, despite the fact that plots at the Andrews Nursery were purposely located in a compartment with a history of charcoal root rot caused by *Macrophomina phaseolina*. In fact, the only indication of any root disease present in our plots was scattered damping-off, apparently caused by species of *Fusarium*, *Pythium*, and *Rhizoctonia*, and possibly other fungi. In the 1993 crop, damping-off caused primarily by *Pythium myriotylum* Drechs. resulted in statistically significant reductions in seedling numbers in check plots compared to methylbromide treated plots (Table 1). However, this difference was not maintained in the 1994 and 1995 crops even though treatment plots were maintained in the same locations throughout the study.

Organic residues used as soil amendments were selected because of their availability and potential to suppress soil-borne pathogens (Hoitink and Fahy 1986; Pokorny 1982). Rates of application were designed to sufficiently load soils typically deficient in organic matter to induce beneficial changes in soil microflora over time. Effects on soil microbiology are still being evaluated. However, pine bark appears generally preferable to other composted materials.

Lack of background root disease in all plots has precluded meaningful evaluation of organic amendments with respect to disease suppression.

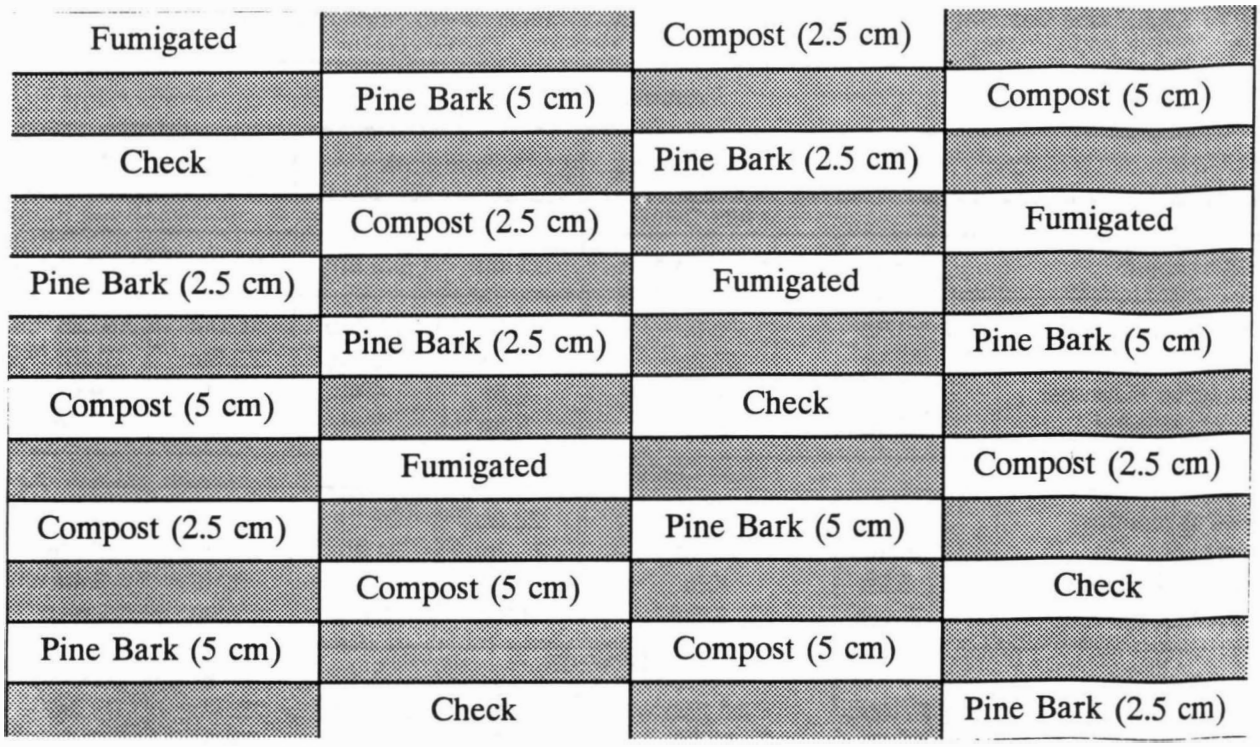

Figure 1. Field layout of study plots at the Florida Division of Forestry's Andrews Nursery in Chiefland, FL. Individual treatment plots are three seedbeds wide (3.7m) by 36.6m long. Fumigated buffers are indicated by shaded areas.

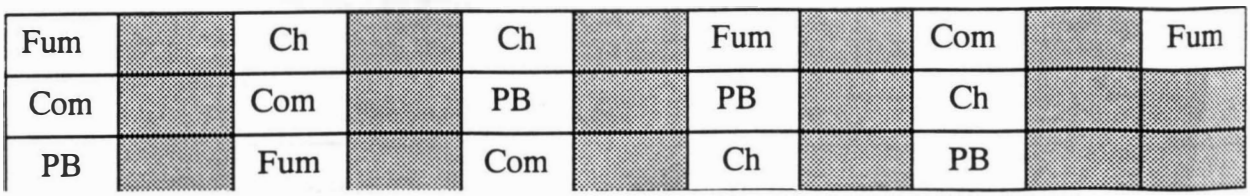

Figure 2. Field layout of study plots at International Paper Company's Supertree Nursery in Blenheim, SC. Individual treatment plots are three seedbeds wide (3.7m) by 12.2m long. Fumigated buffers are indicated by shaded areas (Fum = fumigated; Com = compost 2.5cm; PB = pine bark 2.5cm; Ch = Check).

Table 1. Slash pine seedling production and morphology at the Andrews Nursery, Chiefland, FL[1].

Treatment						
			Pine Bark		Compost	
1993 CROP YEAR						
Measurement[2]	Check	Methyl bromide	2.5 cm	5.0 cm	2.5 cm	5.0 cm
Seedling density	15.3b	20.0a	17.6ab	17.7ab	18.0ab	18.2ab
Height	19.5c	25.2a	21.8bc	22.5ab	23.6ab	21.1bc
Root collar diameter	4.6a	4.9a	4.5a	4.4a	4.7a	4.6a
Shoot/root ratio	2.4a	2.9a	2.8a	2.4a	2.9a	2.5a
1994 CROP YEAR						
Seedling density	10.9a	22.1a	22.2b	22.1a	21.4a	20.4a
Height	24.6c	29.1a	26.2bc	24.9bc	29.3ab	27.9bc
Root collar diameter	5.2a	4.8a	4.8a	5.2a	5.1a	5.4a
Shoot/root ratio	3.1ab	3.5a	3.3ab	3.0bc	3.6a	3.4ab
1995 CROP YEAR						
Seedling density	24.3bc	26.2ab	27.3ab	25.2abc	23.4bc	22.7cb
Height	23.9b	28.6a	25.7bc	23.6b	28.4a	25.7b
Root collar diameter	4.7bc	4.7abc	4.4cd	4.2d	5.2a	4.9ab
Shoot/root ratio	3.6ab	4.3a	3.5b	3.4b	3.8ab	3.5b

[1] Mean seedling counts based on 12 subplot counts per treatment. All other means based on measurements of 160 seedlings per treatment. Treatment means for each variable (rows) followed by the same letter are not signficantly different (P=0.05) according to Duncan's multiple range test.

[2] Seedling density based on number of seedlings per 929cm^2 (1 ft^2); height given in cm; root collar diameter given in mm.

Nevertheless, lack of disease development in both fumigated and non-fumigated plots raises legitimate questions regarding the need for and cost-effectiveness of routine use of methyl bromide for root disease control in these two test nurseries.

Once this project is completed, a final report will be issued which will discuss all the data and our conclusions regarding the issue of methyl bromide fumigation in forest tree nurseries. Even though much of our data are relatively inconclusive, it will provide a substantial and useful baseline for continued discussions and considerations of new approaches.

ACKNOWLEDGEMENTS

The authors express their appreciation to C. Affeltranger, E. Ash, S. Gilly, M. Cram, C. Gramling, International Paper Co. and Jefferson Smurfit Corporation for invaluable support rendered to this effort. Funding for this work was provided by USDA Forest Service, Forest Health, Region 8.

Table 2 Loblolly pine seedling production and morphology at International Paper Company's Supertree Nursery, Blenheim, SC.[1]

Measurement[2]	Treatment: Check	Methyl bromide	Pine Bark 2.5 cm	Compost 2.5 cm
1994 CROP YEAR				
Seedling density	28.8a	26.4ab	24.7ab	23.8ab
Height	33.6ab	35.3a	33.3ab	31.7ab
Root collar diameter	5.0ab	5.1ab	5.4a	4.6a
Shoot/root ratio	3.9a	4.1a	3.5b	3.6b
1995 CROP YEAR				
Seedling density	23.3ab	23.9a	21.4b	24.0a
Height	28.7b	28.6b	27.2bc	31.5a
Root collar diameter	4.2a	4.1a	4.2a	4.4a
Shoot/root ratio	3.2b	3.3b	3.5b	4.2a

[1] Mean seedling counts based on 12 subpolot counts per treatment. All other means based on measurements of 160 seedlings per treatment. Treatment means for each variable (rows) followed by the same letter are not signficantly different (P=0.05) according to Duncan's multiple range test.

[2] Seedling density based on number of seedlings per 929cm² (1 ft²); height given in cm; root collar diameter given in mm.

Table 3. First-year field outplant performance of slash pine seedlings from the Andrews Nursery, Chiefland, FL[1].

Measurement[2]	Treatment: Check	Methyl bromide	Pine Bark 2.5 cm	Pine Bark 5.0 cm	Compost 2.5 cm	Compost 5.0 cm
1993 SEEDLING CROP						
Percent survival	100a	99.2a	99.3a	99.2ab	99.3ab	99.7ab
Height	R6.7ab	53.4b	59.4ab	60.7ab	59.9ab	62.6ac
Root collar diameter	20.7a	19.1a	20.3a	21.9a	20.8a	22.2a
Plot volume index	121.7ab	100.0b	123.6ab	146.ab	129.5ab	157.0a
1994 SEEDLING CROP						
Percent survival	99.a	98.8a	100.0a	99.0a	99.2a	99.4a
Height	51.1a	52.9a	55.4a	53.3ac	55.1a	55.8ac
Root collar diameter	15.1a	14.6a	15.9a	16.4a	16.1a	17.3a
Plot volume index	58.9a	59.5a	72.7a	71.6a	72.2a	83.6a

[1] Survival means based on 12, 50-tree plots per treatment. Mean heights based on 25 seedlings per plot (=300 seedlings per treatment). Mean root collar diameters based on 15 seedlings per plot (=180 seedlings per treatment). Treatment means for each variable (rows) followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

[2] Height given in cm; root collar diameter given in mm; plot volume index = (mean height) x (mean root collar diameter)² x (mean number surviving seedlings/plot).

Table 4. First-year field outplant performance of loblolly pine seedlings from International Paper Company's Supertree Nursery in Blenheim, SC[1].

Treatment				
1994 SEEDLING CROP				
Measurement[2]	Check	Methyl bromide	Pine Bark 2.5 cm	Compost 2.5 cm
Percent survival	97.0a	97.5a	95.5ab	97.0a
Height	52.9a	53.4a	51.4ab	48.8b
Root collar diameter	8.6ab	9.0a	8.2b	8.1b
Plot volume index	19.0ab	21.3a	16.5b	15.7b

[1] Survival means based on 12, 50-tree plots per treatment. Mean heights based on 25 seedlings per plot (=300 seedlings per treatment). Mean root collar diameters based on 15 seedlings per plot (=180 seedlings per treatment). Treatment means for each variable (rows) followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.
[2] Height given in cm; root collar diameter given in mm. Plot volume index = (mean height) x mean root collar diameter)^2 x (mean number surviving seedlings/plot).

LITERATURE CITED

Barnard, E.L., M.E. Kannwischer-Mitchell, D.J. Mitchell and S.W. Fraedrich. 1996. Progress and trends: Southeastern regional component of a national technology development effort evaluating nursery management and seedling development without dependence on methyl bromide. Southwide Forest Disease Workshop, Clemson, SC. Jan. 22-25, 1996. (Abstr.).

Barnard, E.L., M.E. Mitchell, D.J. Mitchell and S.W. Fraedrich. 1994. Evaluating the efficacy of orgnic soil amendments as alternatives to methyl bromide for control of soilborne pathogens in forest tree nurseries. Southwide Forest Disease Workshop, Asheville, NC. June 7-9,1994. (Abstr.).

Civerolo, E.L., S.K. Narang, R. Ross, K.W. Vick and L. Greczy. (eds.). 1993. Alternatives to methyl bromide: assessment of research needs and priorities. Proceedings from the USDA Workshop on Alternatives to Methyl Bromide. Arlington, VA. June 29-July 1, 1993. 85p.

Hoitink, H.A.J. and P.C. Fahy. 1986. Basis for the control of soilborne plant pathogens with composts. Annual Review of Phytopathology 24:93-114.

James, R.L., D.M. Hildebrand, S.J. Frankel, M.M. Cram and J.G. O'Brien. 1993. Alternative technologies for management of soil-borne diseases in bareroot forest nurseries in the United States. *In*: Landis, T.D. (tech. coord.). Proceedings: Forest and Conservation Nursery Associations. USDA Forest Service, General Technical Report GTR-RM-257. pp. 91-96.

Kannwischer-Mitchell, M.E., E.L. Barnard, D.J. Mitchell and S.W. Fraedrich. 1994. Organic soil amendments as potential alternatives to methyl bromide for control of soilborne pathogens in forest tree nurseries. *In*: Landis, T.D. and R.K. Dumroese (tech. coords.). Proceedings: Forest and Conservation Nursery Proceedings. USDA Forest Service, General Technical Report GTR-RM-257. pp. 12-15

Porkorny, F.A. 1982. Pine bark as a soil amendment. *In*: Brissette, J. and C. Lanz (compilers). Proceedings: 1982 Southern Nursery Conferences. USDA Forest Service. Technical Publication R8-TP4. pp. 131-139.

Singleton, L.L., J.D. Mihail and C.M. Rush (eds.). 1992. Methods for Research on Soilborne Phytopathogenic Fungi. APS Press, St. Paul, MN. 265p.

Smith, R.S., Jr. and S.W. Fraedrich. 1993. Back to the future - pest management without methyl bromide. Tree Planters' Notes 44:87-90.

BURKHOLDERIA CEPACIA: A MICROBIAL INOCULANT WITH COMMERCIAL POTENTIAL FOR BIOLOGICAL CONTROL OF FUNGAL PATHOGENS IN FORESTRY[1]

L.M. Funk[2], D.N. He[2], E.A. Pedersen[2] and M.S. Reddy[2]

ABSTRACT

Fungal pathogens are widespread in forest nurseries, as well as on reforestation sites. Severe pre- and post-emergence damping-off may result in a 15-25% reduction in number of harvestable seedlings in conifer nurseries. Furthermore, a large percentage of seedlings planted on reforestation sites fail to establish, or experience "growth check" partially due to pathogenic fungi in forest soils. Agrium Inc. is researching a naturally-occurring soil bacterium, *Burkholderia cepacia* strain Ral-3, for use as a seed treatment and a seedling root dip in the forest industry. The inoculant is produced in a commercial liquid formulation which is stable at various storage temperatures. When used as a seed treatment, the strain suppresses damping-off and root rot pathogens by aggressively colonizing emerging roots of various conifer species. *In vitro* assays showed that strain Ral-3 significantly suppressed hyphal growth of several potentially-pathogenic fungi, including species of *Pythium*, *Rhizoctonia*, *Fusarium*, and *Cylindrocarpon*. Replicated laboratory assays revealed that various conifer seeds treated with strain Ral-3 gave significantly improved healthy seedling stands compared to nontreated controls after seeding into growth mix artifically infested with *Fusarium*, *Pythium* and *Rhizoctonia*. In a commercial conifer nursery, emergence of Douglas-fir seed treated with Ral-3 was significantly improved compared to nontreated seed. Additional tests as a seedling root dip on various seedlings showed that Ral-3 treated seedlings had significantly increased new shoot and root biomass compared to non-inoculated control seedlings. Other aspects of product development were also discussed. From these results, it is evident that strain Ral-3 has commercial potential for use as a biological control agent in the forest industry.

[1] Paper presented at the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). Orlando-Gainesville, FL., USA. May 19-24, 1996. Only the abstract is available; completed paper was not provided for proceedings.

[2] Agrium, Inc., Saskatoon, Saskatchewan, Canada.

ALTERNATIVES TO METHYL BROMIDE SOIL FUMIGATION IN A LODGEPOLE PINE NURSERY: EXPERIMENTS WITH BARE FALLOWING, SOLARIZATION AND COVER CROPS, ALONE AND COMBINED WITH BIOCONTROL SEED DRESSINGS[1]

M.J. Peterson[2] and J.R. Sutherland[2]

ABSTRACT

An experiment was conduted in a bareroot, lodgepole pine (*Pinus contorta*) nursery in south-central British Columbia, Canada, to find alternatives to methyl bromide soil fumigation. Seeds were treated with one of three biocontrol agents, i.e., Polyversum, Supresivit or Ral-3 and sown in soils: (i) fumigated with methyl bromide, (ii) into which a pea-oats cover crop was incorporated, (iii) subjected to solarization, (iv) bare fallowed plus cultivation, and (v) bare fallowed without cultivation (control). Data were collected on populations of soil-borne species of *Fusarium*, *Pythium* and *Trichoderma*, germination/seedling survival, and percentage packable (saleable) seedlings. The results showed that by year 2, cover cropping favored buildup of both *Fusarium* and *Pythium*. Populations of *Trichoderma* while high, especially following soil fumigation, decreased steadily in all treatments through to the end of year 2. Germination/seedling survival was comparable in the fumigated, bare fallow plus cultivation and solarization plots, and less but equal in the control (bare fallow without cultivation) and cover crop treatments. Weed populations were lowest and equal in the methyl bromide and bare fallow plus cultivation plots and high and equal in the remaining treatments. Temperatures in the soil solarization treatment did not reach high enough levels to adequately control pathogens or weeds. Effects of seed treatments were variable, however, Polyversum and Ral-3 showed some benefit in the methyl bromide and control, and bare fallow plus cultivation treatments, respectively. The percent of packable seedlings was highest in the methyl bromide treatment and equal in all other treatments. Based on the control of potentially pathogenic fungi, numbers of surviving seedlings, and percentage packable seedlings, we feel that bare fallowing plus cultivation is a viable alternative to methyl bromide soil fumigation. This is especially true once cultural practices such as soil fertilization are modified to increase numbers of packable seedlings.

INTRODUCTION

Studies in the early 1950's amd 1960's, especially by Foster (1961) and Henry (1953) in the southern U.S.A., showed the benefits of using methyl bromide soil fumigation against soil-borne diseases, insects, and weeds in forest nurseries. Since then this practice has become widespread in the U.S.A., e.g., in the upper midwest (Anderson et al. 1956), across the South (Fraedrich 1994 and references therein), California (Krugman et al. 1965), in the Rocky Mountain area (Boyd 1971) and elsewhere for several widespread root and soil-borne diseases (Peterson and Smith 1975), and in Canada, especially in the east (Sutherland 1967). However, resulting from the 1991 Montreal Protocol Assessment, methyl bromide will no longer be available for use in North America after the year 2000 (James et al. 1994). Thus, alternatives to methyl bromide soil fumigation are being sought for use in forest nurseries. Examples of alternatives being considered include using other chemicals such as fungicides and herbicides, crop rotation and fallowing and other cultural practices, physical methods which include heat pasteurization and solarization, and biological control, e.g., using beneficial microorganisms, bioactive composts, and bioherbicides (Linderman et al. 1994). In British Columbia (B.C.), the need to find alternatives to methyl bromide soil fumigation is not nearly as great as elsewhere since most seedlings are grown in containers using peat-based media which are ordinarily free of most pests and weeds. However, there will be some effect of banning methyl bromide use as some seedlings and transplants are still grown in bareroot nurseries.

[1] Paper presented at the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). Orlando-Gainesville, FL., USA. May 19-24, 1996.

[2] Applied Forest Science Limited, Victoria, B.C., Canada.

The purpose of this study was to compare efficiency of alternative soil treatments and seed-applied biocontrol products, alone and combined, with that of methyl bromide against soil-borne diseases, particularly damping-off, and weeds. Data were also collected on seedling growth and numbers of packable seedlings.

MATERIALS AND METHODS

The experiment was conducted from 1994-1995 at Weyerhauser Canada's Grandview Nursery, near Armstrong, B.C. (50° 23' 10" N 119° 18' 10" W). The soil there is a sandy loam (pH ca 6.0, 1.2% organic matter). On May 2, 1994, the soil was rototilled to about 30cm in depth, then at various dates thereafter the following soil treatments were applied: (i) methyl bromide (MC-33) at 440 Kg/ha on September 24 after rototilling the soil fortnightly since May 10, the treated soil was covered and sealed with a 1 mil-thick, clear plastic which was removed 5 days following treatment, (ii) cover crop (50% peas, 50% oats) sown May 10, soil rototilled just before forming the seedbeds, (iii) bare fallow plus cultivation wherein the soil was rototilled fortnightly from May 10 to September 24, then left undisturbed until the seedbeds were formed the following spring, (iv) soil solarization in which the soil was rototilled on June 10 and then covered with a clear 1 mil-thick, plastic cover which was removed on May 5, 1995; throughout this period soil temperatures were recorded at two localities at the soil surface and at 15 and 25cm depths using thermisters and a Campbell model CR-21 data logger (Campbell Scientific, Logan, UT), and (v) control (fallow soil, no cultivation/rototilling).

The seeds used were lodgepole pine, *Pinus contorta* Dougl. ex Loud. [British Columbia Ministry of Forests (BCMoF) seedlot No. 27056 which had a germination capacity of 96%]. They were stratified (as per ISTA rules) and sown in 6 rows at 120 seeds/linear m of seedbed in the soil treatment plots on May 23, 1995, after treatment (maximum amount adhering) with one of the following commercial products (main ingredient in parentheses): Supresivit (*Trichoderma harzianum* Rifai), Polyversum (*Pythium oligandrum* Drechsler) or Ral-3 (*Burkholderia cepacia* strain Ral-3).

Each soil treatment X seed treatment combination was replicated three times in 3 X 25m plots in a completely random design. On May 11, July 15, September 20 and November 15, 1994, and May 2, June 30 and September 30, 1995, 20-25 soil samples (2cm wide, X 23cm long soil cores/sample) were collected at about 1m intervals from each replicate of each treatment and after the cores within each replicate were mixed, the soils were assayed using selective media for species of *Fusarium* (Komada 1975), *Pythium* (Hamm and Hansen 1990) and *Trichoderma* (Papavizas and Lumsden 1982).

To determine germinant emergence and seedling survival, seedling counts were made 6 weeks after seed sowing in spring, 1995 using a randomly-selected $1m^2$ subplot for each treatment plot. In some instances, weeds were too numerous to count. Thus, weed populations were estimated by collecting the shoots of all weeds in a randomly selected $1m^2$ subplot in each treatment plot. The shoots were then dried at 35°C for 24 hours and weighed. On January 10, 1996, the seedlings were lifted and graded as packable (meeting standards such as height and root collar diameter) or culls according to BcmoF standards (Scagel et al. 1993)

The data for the various parameters were subjected to analysis of variance (ANOVA) and the significance of mean differences determined using Duncan's multiple range test at $P=0.05$. The data for populations of soil-borne *Fusarium*, *Pythium* and *Trichoderma* were subjected to a one-way ANOVA up until the seeds were sown on May 23, 1995, and thereafter to a multi-way ANOVA. percentage packable seedling data were transformed to arcsin for statistical analysis.

RESULTS

Soil Fungi:

Since the analysis of variance showed no discernible effects of any of the seed treatments on population levels, or changes, of soil-borne *Fusarium*, *Pythium* or *Trichoderma* we have shown only the soil treatment effects over time on these fungi (Figure 1). *Fusarium* levels were quite low in all but the cover crop and control treatments over the two season sampling period. The highest *Fusarium* populations occurred in the cover crop treatment in November, 1994, and July, 1995 (Figure 1). Following methyl bromide fumigation, *Pythium* populations in these soils dropped to undetectable levels of <10 cfu (colony-forming units per gram oven-dry soil) of soil (Figure 1). While there were no such dramatic reductions in *Pythium* numbers in the bare fallow and solarization treatments, overall *Pythium* levels did not differ in these soils from those in fumigated soils. Only in the cover crop and control treatments were *Pythium* numbers significantly higher (Figure 1).

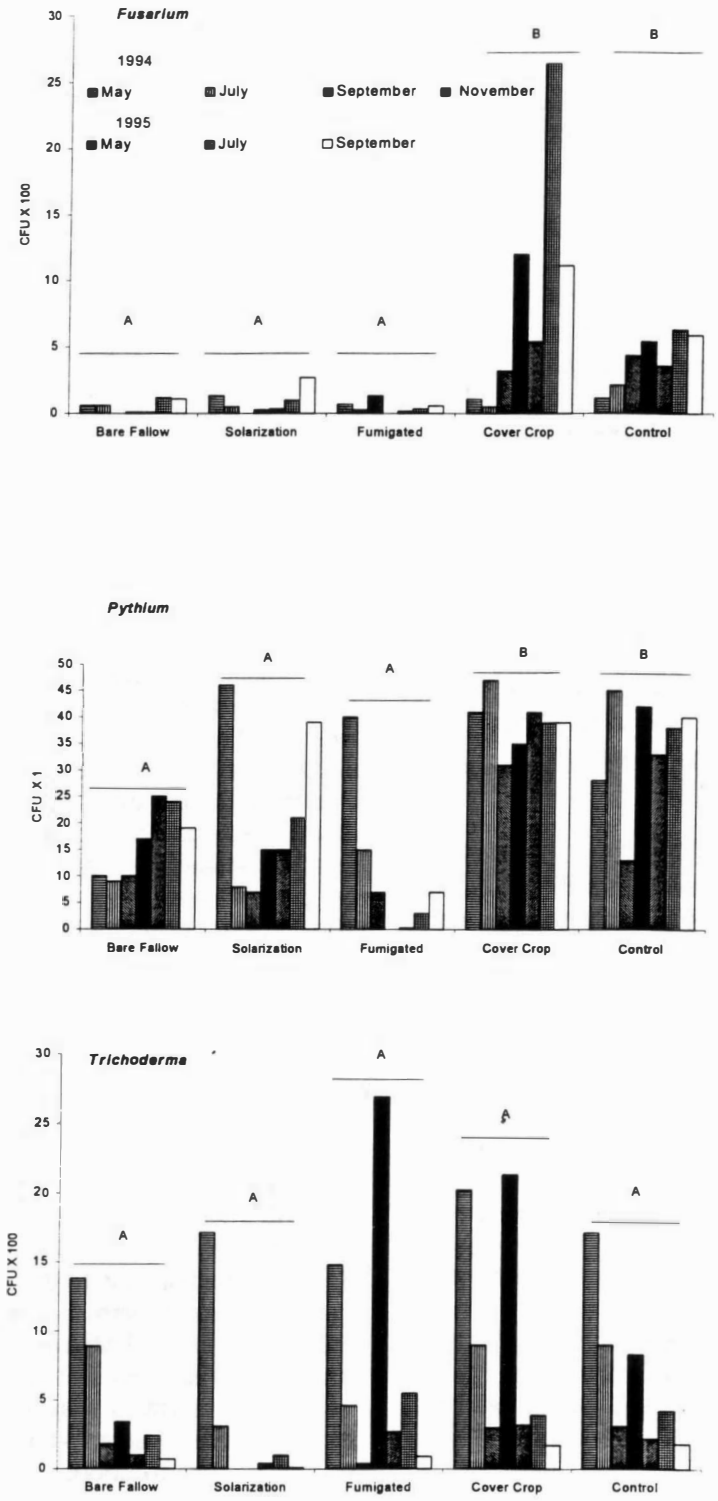

Figure 1. The main effects of soil treatments on populations of soil-borne species of *Fusarium*, *Pythium* and *Trichoderma* (top to bottom) over the 1994-95 seasons. Values groupd by the same letter are not significantly different (P=0.05). CFU=colony-forming units per gram oven-dry soil.

Immediately following soil fumigation, *Trichoderma* populations increased dramatically and, although they appeared to decrease steadily in all treatments over time, none of the differences were significant owing to the large variations in *Trichoderma* numbers at each sample date.

Soil Temperatures - Solarization Plots:

Figure 2 shows the mean (based on the high and low temperatures for each hour), daily air, and soil (surface, and 15 and 25 cm depths) temperatures for July and August, 1994 (in the season before seeds were sown). Temperatures are only given for this period because this was when they were highest. In general, temperatures followed the expected diurnal pattern of increasing in the morning, peaking about 15:00, then declining through the evening and night. Soil temperatures beneath the plastic covering were usually higher than air temperatures, especially at the soil surface where temperatures reached almost 5°C between 15:00 and 17:00. Daily temperatures at 25 cm depth remained almost constant over the 2-month sampling period.

Germinants:

Germinant emergence and seeling survival counts showed that for the main effects of the soil treatments, across all seed treatments, there were no significant differences in seedling numbers among the fumigated, solarization, and bare fallow treatments. However, there were significantly fewer, and equal, numbers of seedlings in the control (bare fallowed without cultivation) and cover crop treatments (Figure 3, capital letters). The soil treatment X seed treatments results (Figure 3, lower case letters) showed that there were fewer seedlings in the bare fallow, solarization and methyl bromide plots when sown seeds were either untreated or treated with Supresivit (first two of these soil treatments) or with Supresivit or Ral-3 in fumigated soils. Polyversum seed treatment resulted in fewer seedlings in the cover crop treatment while seed treatment had no effect on seedling numbers in the control soils.

Weeds:

Based on their dry weight, weed populations were highest, and not significantly different (P=0.05) in the solarization, cover crop and control treatments, and lowest, but not significantly different (P=0.05) in the bare fallow and methyl bromide fumigation plots (Figure 4).

Packable Seedlings:

Considering only the main effects of soil treatments (Figure 5, capital letters), the percentage of packable seedlings were significantly greater (P=0.05) in the fumigated-soil plots and not significantly different among any of the other soil treatments. Numbers of seedlings lifted averaged about 11,100 per plot (range 3,000-13,500). Within the fumigated soil treatment, the most packable seedlings occurred in plots sown with Polyversum and untreated seeds, while numbers of such seedlings were lower and equal in plots receiving Supresivit and Ral-3-treated seeds (Figure 5, lower case letters). There were no consistent effects of seed treatment on numbers of packable seedlings in any of the other treatments.

DISCUSSION

This research determined that other soil treatments or applications of biocontrol agents to seeds, alone or combined, can be used as alternatives to methyl bromide soil fumigation for managing diseases and weeds in a bareroot, lodgepole pine nursery in south central B.C. The major consideration was treatment effects on numbers of packable (plantable) seedlings which could be used for reforestation. Regarding the effects of various soil treatments on potentially-pathogenic, soil fungi, our results showed that *Fusarium* populations were consistently higher in the cover crop treatment, especially after it was plowed in, and the non-cultivated bare fallow treatment. Higher *Fusarium* populations following use of cover crops has been noted before in B.C. (Sutherland, Dennis and Lock unpublished) and Oregon (Hansen et al. 1990). The higher *Fusarium* populations in the non-tilled (control) as compared to the tilled, bare fallow soils showed the importance of tilling such soils to reduce *Fusarium* levels. Our finding that bare fallow plus cultivation reduced *Fusarium* populations to the same levels as methyl bromide agrees with the results of Stone et al. (1997). The one possible advantage of using methyl bromide fumigation is that *Pythium* populations, which over all dates were lower in the bare fallow plus cultivation, solarization and methyl bromide treatments, fell to undetectable levels following fumigation. Although they rebounded somewhat, populations never reached the levels of other treatments. However, *Pythium* is seldom a problem in our nurseries unless soil moisture remains consistently high. Except for the increase following fumigation, *Trichoderma* levels declined in all treatments over the two sampling seasons. The post-fumigation increase in *Trichoderma* numbers has

been reported earlier (Bloomberg 1965; Sutherland et al. 1966).

Our germinant survival and weed control data showed that methyl bromide fumigation provided no advantage over bare fallowing plus cultivation for controlling weeds. Perhaps solarization would have provided better weed and pathogen control if we would have used two layers of plastic covering which increases soil temperature where climatic conditions are suboptimal for single - layer solarization (Annesi et al. 1994). Our results showed that only temperatures at the soil surface reached levels approaching 50 °C which would provide some weed and pathogen control. Soil fumigation increased numbers of packable seedlings, particularly when the seeds were treated with Polyversum. However, packable seedling numbers were comparable when untreated seeds were sown in fumigated and bare fallow, cultivated soils, about 50 and 55% of the seeding crop in the two treatments, respectively. Also, methyl bromide fumigation gave no better control of potentially pathogenic *Fusarium* and *Pythium* and weeds than did bare fallowing plus cultivation. We feel that problems associated with packable seedlings in bare fallow plus cultivation can be overcome by changing fertilizer or sowing density regimes which are currently geared toward growing seedlings in fumigated soils.

In conclusion, we see no major reasons why bare fallowing with summer cultivation cannot replace methyl bromide soil fumigation at the Grandview Nursery. Fallowing with cultivation readily fits into the existing nursery schedule; following fall seedling lifting, fields are left fallow, or sown to a cover crop, until soil temperatures are high enough the following late summer to early fall to fumigate with methyl bromide. It is during this summer period when soils could be bare fallowed and cultivated. Problems such as depletion of soil organic matter without using a cover crop, could be overcome by amending fields with peat or composted sawdust purchased with funds now spent on fumigation. Indeed, use of cover crops which stimulates potential pathogens, undoubtedly contributes to the need for soil fumigation. Besides concerns about its relationship to ozone depletion, some other problems associated with methyl bromide use in forest nurseries are its high cost, health risks to humans and elimination of mycorrhizal fungi.

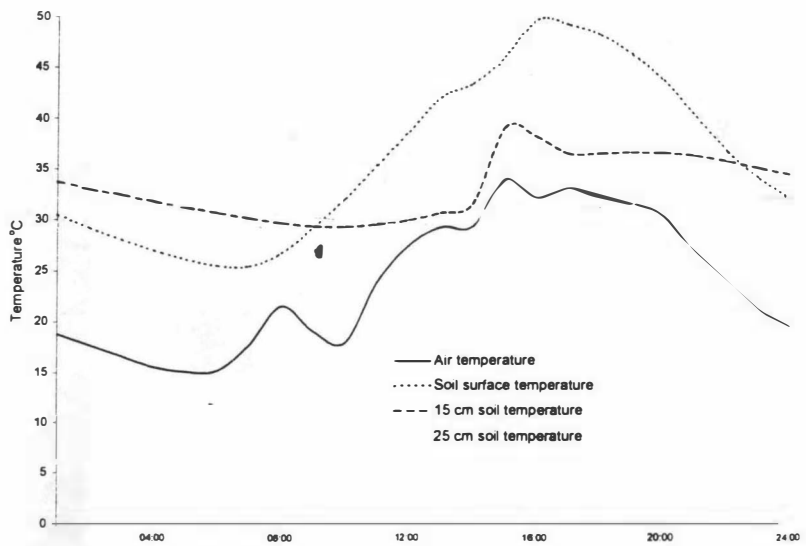

Figure 2. Mean (average of the high and low for each hour) daily soil temperatures beneath the plastic cover during July through August 1994, for two locations in the soil solarization plots. Air temperature was recorded 50cm above the cover.

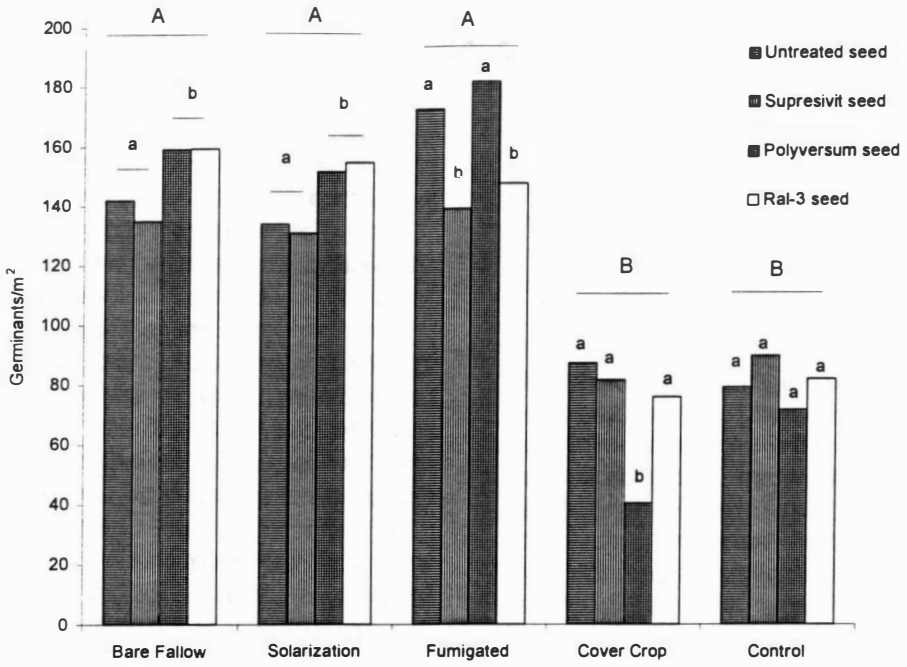

Figure 3. Numbers of lodgepole pine germinants in plosts 6 weeks after seed sowing. Soil treatment effects (groups of four columns) and seed treatment effects (any of the four columns within each soil treatment) are not significantly different (P=0.05) is grouped by the same capital letter or lower case letter, respectively.

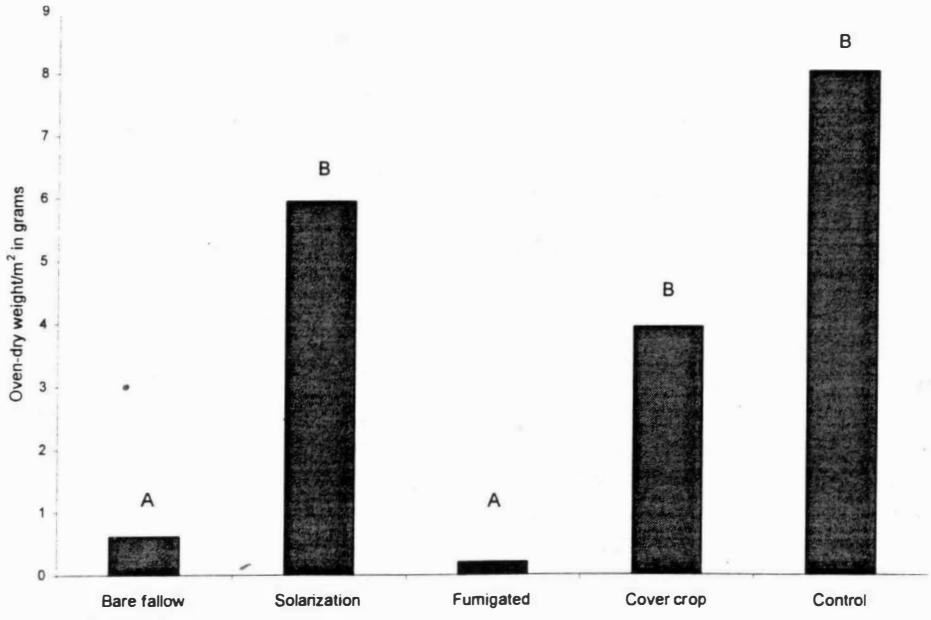

Figure 4. Weight of weeds by soil treatment in early summer 1995, or about 13 months post-treatment. Columns with the same letter are not significantly different (P=0.05).

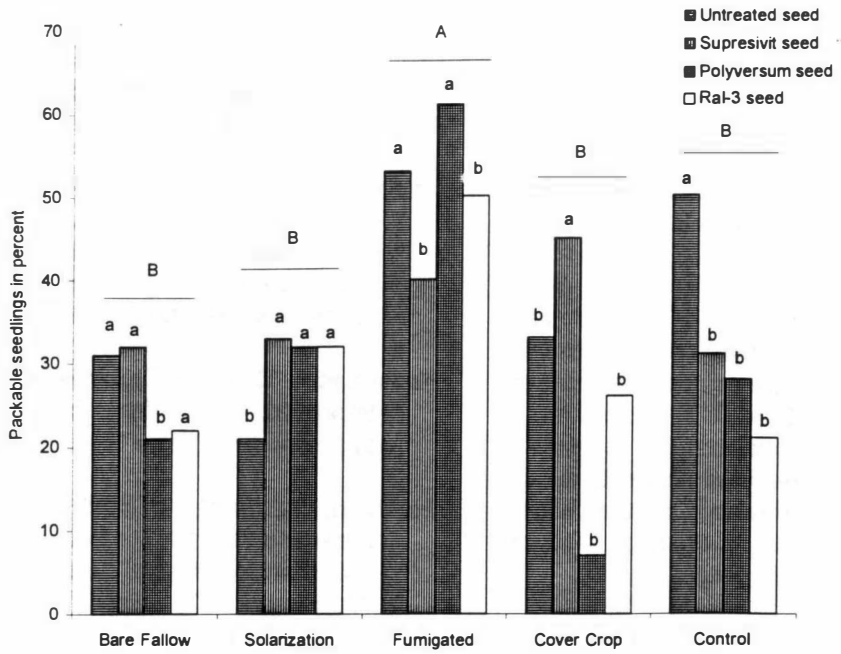

Figure 5. percentages (based on numbers lifted) of pine seedlings in the plots when lifted in January, 1996. Soil treatment effects (groups of four columns) and seed treatment effects (any of the four columns with each soil treatment) are not significantly different (P=0.05) if grouped by the same capital letter or lower case letter, respectively.

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LITERATURE CITED

Anderson, R.L., D.D. Skilling and E.D. Clifford. 1956. Nursery root disease control by soil fumigation. Technical Notes No. 449, Lake States Forest Experiment Station. USDA Forest Service, St. Paul, MN. 1p.

Annesi, T., E. Motta and M. Marchionni. 1994. A two year study on solarization of forest nursery soil. *In*: Perrin, R. and J.R. Sutherland (eds.). Proceedings of the third meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Institut National De La Recherche Agronominque. Paris, France. Les Colloques No. 68. pp. 221-225.

Bloomberg, W.J. 1965. The effect of chemical sterilization on the fungus population of soil in relation to root diseases in Douglas-fir seedlings. Forestry Chronicle 41:182-187.

Boyd, R.J. 1971. Effects of soil fumigation on production of conifer nursery stock at two northern Rocky Mountain nurseries. USDA Forest Service Research Paper INT-91. 19p.

Foster, A.A. 1961. Control of black root rot of pine seedlings by soil fumigation in the nursery. Georgia Forest Research Council Report No. 8. Southeastern Forest Experiment Station, Macon, GA. 5p.

Fraedrich, S.W. 1994. Soil fumigation in southern forest tree nurseries: current status and future needs for pest management. *In*: Perrin, R. and J.R. Sutherland (eds.). Proceedings of the second meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Institut National De La Recherche Agronominque, Paris, France. Les Colloques No. 68. pp. 267-282.

Hamm, P.B. and E.M. Hansen. 1990. The isolation and identification of *Phytophthora* species causing damage in bare-root conifer nurseries. *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada. Pacific and Yukon Regions. Information Report BC-X-331. pp. 169-179.

Hansen, E.M., D.D. Myrold and P.B. Hamm. 1990. Effects of soil fumigation and cover crops on potential pathogens, microbial activity, nitrogen availability, and seedling quality in conifer nurseries. Phytopathology 80: 698-704.

Henry, B.W. 1953. A root rot of southern pine nursy seedlings and its control by soil fumigation. Phytopathology 43:81-88.

James, R.L., D.M. Hildebrand, S.J. Frankel, M.M. Cram and J.G. O Brian. 1994. Alternative technologies for management of soil-borne diseases in bare root forest nurseries in the United States. *In*: Perrin, R. and J.R. Sutherland (eds.). Proceedings of the second meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Institut National De La Recherche Agronominque, Paris, France. Les Colloques No. 68. pp. 237-246.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review Plant Protection Research (Japan) 8:114-125.

Krugman, S.L., E.C. Stone and R.V. Bega. 1965. The effects of soil fumigation and lifting date on the root - regenerating potential of Monterey pine planting stock. Journal of Forestry 63: 114 - 119.

Linderman, R., W. Dixon, S. Fraedrich and R.S. Smith, Jr. 1994. Alternatives to methyl bromide: Assessment of research needs and priorities for forestry, nursery, and ornamental crops. Tree Planters' Notes 45:43-46.

Papavizas, G.C. and R.D. Lumsden. 1982. Improved medium for isolation of *Trichoderma* spp. from soil. Plant Disease 66:1019-1020

Peterson, G.W. and R.S. Smith, Jr. 1975. Forest nursery diseases in the United States. Agricurtural Handbook No. 470, USDA Forest Service, Washington D.C. 125p.

Scagel, R., R. Bowden, M. Madill and C. Kooistra. 1993. Provincial seedling stock type selection and ordering guidelines. Silviculture Branch, B.C. Ministry of Forests, Victoria, Canada. 75p.

Stone, J.K., D. Hildebrand, R.L. James and S.M. Frankel. 1997. Alternatives to chemical fumigation in bareroot forest nurseries: effects on pathogen levels and seedling density, mortality and quality. *In*: James, R.L. (ed.). Proceedings of the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). USDA Forest Service, Northern Region, Forest Health Protection pp. 48-58.

Sutherland, J.R. 1967. Field tests for the control of red pine seedling diseases. Phytoprotection 48:58-67.

Sutherland, J.R., R.E. Adams and R.P. True. 1966. *Pythium vexans* and other conifer seedbed fungi isolated by the apple technique following treatments to control nematodes. Plant Disease Reporter 50:545-547.

MISCELLANEOUS PAPERS

GREMMENIELLA ABIETINA: **CONIDIAL AND MYCELIAL ASPECTS**[1]

R. L. Petaisto[2], M. L. Juntunen[2] and E. O. Kajander[3]

ABSTRACT

Gremmeniella abietina is a pathogenic ascomycete causing damage especially in pines. Fungicides are used to control this disease in forest nurseries. Differences between conidia and mycelia in protein, fatty acid and sterol composition and immunogenicity were studied in preliminary experiments. Results indicated that although the protein patterns of conidia and mycelia were closely related, immunologically they differed. However, the double band in Western blot characteristic for B-type mycelial samples was also found in conidial samples of B-type. Fatty acids and sterols differed quantitatively between conidia and mycelia. Chemical control with chlorothalonil (Bravo 500®) was effective only around the time of spore germination. The observed differences should be of value in diagnostics and control of this fungus.

INTRODUCTION

Gremmeniella abietina (Lagerb.) Morelet, cause of Scleroderris canker, is a pathogenic ascomycete found in Europe, northern North America and northern Asia. It causes damage to conifers especially in the genus *Pinus*. Damage occurs to seedlings and older trees. Cool and rainy summers predispose trees to the disease. The European race in Europe can be divided into at least two types: A-type (large tree type) and B-type (small tree type) (Hellgren and Hogberg 1995; Müller and Uotila 1997; Uotila 1983). These types can be identified with immunoblotting using mycelial samples (Petaisto et al. 1996). Conidia and ascospores have different times of dispersal; conidia are dispersed mainly in the beginning of summer and ascospores in late summer. Disease symptoms will become visible the following spring.

During growth phase, pine shoots are especially susceptible to infection (Petaisto and Kurkela 1993).

Scots pine seedlings, older than 1 year, stop their stem growth at heat sum about 500 d.d. and the needles a little later (Koski and Sievanen 1985), but first-year seedlings continue their growth into late summer. In Finnish forest nurseries the production of 1-year-old container seedlings is currently common; seedlings are grown under plastic the first half of the growing season. Earlier, production of 2-year-old bareroot seedlings was more common. Therefore, control procedures were changed to accomodate container-grown seedlings.

Earlier studies on *G. abietina* infection were usually made using seedlings older than 1 year. To our knowledge, only Hamnede (1980) and now we have carried out infection experiments with first year seedlings. Because conditions for seedling production in forest nurseries have changed, we needed basic knowledge about fungus-host relationships to improve disease control in nurseries.

G. abietina grows quite slowly. Spore germination and hyphal penetration into the host occurs slowly. For purposes of disease control, possible similarities and differences between spores and mycelia were examined. In this paper, possible similarities and differences of *G. abietina* conidia and mycelia in fatty acid and sterol composition, in proteins and antigens, and in chemical control were evaluated.

MATERIALS AND METHODS

SDS-PAGE protein pattern and immunoblot:

For mycelial samples, isolates were cultured two weeks in V8-liquid medium and for conidia production in barley corn and Scots pine needle homogenate (Petaisto and Kurkela 1993; Petaisto et al. 1994). Soluble proteins were extracted from mycelia and conidia and prepared for electrophoresis as described by Petaisto et al. 1994. Protein samples,20g for each pattern, were subjected to SDS-PAGE on 14% gels and gels were stained after the run with Coomassie brilliant blue. Conidial samples were compared to mycelial samples of the same isolates; altogether 12 isolates were tested (Table 1).

[1] Paper presented at the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). Orlando-Gainesville, FL., USA. May 19-24, 1996.

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Polyclonal antibodies against mycelial homogenate of Finnish Toro isolate were produced in hen (Petaisto et al. 1996) and similarly against conidia of the same Toro isolate. The first immunization of the hen was done intramuscular with about 37 million conidia in Freund's complete adjuvant. Thereafter about 7 million conidia in Freund's incomplete adjuvant were given twice followed by similar doses without adjuvants; altogether there were 8 immunizations at 1- to 2-week intervals. The immunoglobulins were isolated from egg yolk with the water dilution method (Akita and Nakai 1993; Kokko et al. 1994).

Western blotting was used to compare antigens present in conidia and mycelia of 12 isolates (Table 1).

Fatty acid and sterol analysis:

Five isolates (Table 1) were grown for fatty acid and sterol analysis on two media: potato-tomato-agar and orange-malt-agar as described by Müller et al. 1994. The conidia were produced in solid media barley corn and Scots pine needle homogenate (Petaisto and Kurkela 1993). Mycelial and conidial samples were homogenized in liquid nitrogen as described by Müller et al. (1994) and lyophilized. The fatty acids and sterols were analyzed by gas chromatography and their amounts were expressed as percent of the total amount of fatty acids and sterols (Müller et al. 1994; Müller and Uotila 1997). This compositional data was not analysed statistically because of the small number of samples.

Table 1. *Gremmeniella abietina* isolates used for fatty acid, sterol and protein analysis and immunoblotting.

Isolate	Isolate Designation[1]	Host[2]	Year of Isolation	Type	Country of Origin
1	Kai 1.2[3,4]	PS	1992	B	Finland
2	Au 45 M.1[4]	PS	1993	A	Finland
3	Au 56 Suo 2.1[4]	PS	1993	B	Finland
4	Au 57 PAT2.1[3]	PS	1993	B	Finland
5	Au 58 PAT2.2[3]	PS	1993	B	Finland
6	Au23/8SUP1 1x`.5[3]	PS	1991	B	Finland
7	Kemijarvi[4]	PS	1992	B	Finland
8	B2[4]	PS	1994	A	Finland
9	12[4]	PS	1992	B	Finland
10	9[4]	PS	1992	B	Finland
11	Hedmark[4]	PS	198-	-	Norway
12	Fsh8B[4]	PC	1990	A	Sweden
13	Ti2L2[4]	PC	1990	A	Sweden
14	Frh6B[4]	PC	1990	A	Sweden
15	R239A3[4]	PC	1991	B	Sweden

[1]Isolates 1-6 were from A. Uotila, isolates 7-10 from J. Kaitera, isolate 11 from M. Vuorinen and isolates 12-15 from M. Hellgren.
[2]PS = *Pinus suylvestris*; PC = *Pinus contorta*.
[3]Isolates used in fatty acid and sterol analysis.
[4]Isolates used in protein pattern and immunoblotting.

Chemical control:

Scots pine seeds were sown on May 9, 1994 in Vapo containers (Parviainen and Tervo 1989) within a plastic greenhouse according to the growing program of Su1njoki Research Nursery. Seedlings were removed from the greenhouse on July 13 and used in an experiment of chemical control of *G. abietina*. Seedlings grown in Vapo containers are normally root pruned in early August. However, no root pruning was done in this experiment.

In each Vapo container tray (40cm x 60cm x 8cm) there were 96 seedlings in rows corresponding to 384 seedlings per m^2. Fifty container trays were used in this experiment. Half of the trays were inoculated on July 18 and other half on August 23. In each tray, seedlings were placed into eight groups, each consisting of eight seedlings. Four groups per tray were inoculated.

Conidia of Finnish *G. abietina* isolates Kai1.2, AU23/8 and AU58 were used in inoculations. The total density of conidia in water suspension was

10^6/ml. 150L of suspension was placed with a micropipet on the growing tip of each inoculated seedling. For two days following inoculation, the surface of seedlings was moistened sprinkler water.

Control treatments using chlorothalonil (Bravo 500®) on seedlings inoculated in July were done either 3 days before or 3 or 12 weeks after inoculation. On seedlings inoculated in August, fungicide treatments were made either 3 days before or 3 or 8 weeks after inoculation. Five container trays were used for each chlorothalonil application. Each chlorothalonil treatment consisted of 160 seedlings.

Chlorothalonil application (2kg a.i./ha; 0.5% water suspension) were made using a special spraying system, with which it was possible to spray 1 container tray (40cm x 60cm) at a time. This system used a Teejet 11003 VB nozzle, similar to tractor or irrigation boom spraying. The spraying pressure was adjusted to between 2.5-3.0 bar for spraying 1200ml per minute (400L/ha). Fungicide was sprayed 50cm above the top of seedlings. Non-fungicide check treatments consisted of water application (pH 7.12 - 7.45, EC 40 - 80 S/cm).

In the spring 1995, seedlings were classified according disease severity. Data were analysed using SYSTAT-software (Systat 1992) with 1 way analysis of variance and Tukey's test.

RESULTS

SDS-PAGE protein pattern and immunoblot:

Only a few differences between the protein patterns of conidia and mycelia were found. Protein bands lower than 14 kD found in conidia were weak or lacking in mycelia. All the bands of higher molecular weight than 14 kD were found both in conidial and mycelial protein patterns. The 20 kD band had a much higher intensity in conidia. 40 kD band had lower intensity in conidial pattern than in the mycelial pattern. (Figure 1a).

The antibodies against conidia or mycelial homogenate both recognized conidial and mycelial proteins in Western blots (Figures 1b and 1c). There was a principal similarity in immunoblots between conidia and mycelia but also differences in intensity and existence of some bands. For example, at about 40 kD there was a double band in mycelial pattern and a single band in conidial pattern when anti-conidia antibodies were used (Figure 1b) and an 20 kD band was stronger in conidial pattern than in mycelial pattern when anti-mycelia antibodies were used (Figure 1c).

The strong 26-28 kD double band typical for B-type in mycelial samples (Petaisto et al. 1996) was also found in conidial samples (Figures 1b and c, lanes 5 and 7). Antibodies against mycelia and conidia both gave the double band in B-type, but the lower part of this double band was weaker when antibodies against conidia were used.

Fatty acids and sterols:

Three fatty acids were clearly most abundant and comprised over 75% of the total amount of fatty acids and sterols. These were hexadecanoic acid (16:0), cis-9,12-octadecadienoic acid (9, 12-18: 2) and cis-9-octadecenoic acid (C18:1) including possible presence cis-9,12,15-octadecatrienoic acid (Figure 2). The percent proportion of this last was smaller (mean=15.9, SD=1.86, n=5) in conidia than in mycelia (mean=32.7, SD=7.08, n=5). The two most abundant occurring sterols were ergos-22-en-3-on and ergosta-5,7,22-trien-3-ol. Percent proportion of both these was larger in conidia (means=5.8 and 8.0, SD=0.89 and 1.41, respectively, n=5) than in mycelia (means=0.74 and 5.03, SD=0.74 and 0.61, respectively, n=5) (Figure 2).

Control of the fungus with fungicide:

The July and August inoculations caused disease in 30% and 79% of the seedlings, respectively (treatment I, Figures 3 and 4). There were significant differences ($P<0.05$) between treatment results in both inoculation group. In July, chlorothalonil application 3 days before inoculation decreased the proportion of diseased seedlings compared to those without chemical control ($P=0.04$, Tukey's test, Figure 3). Chemical controls made later (treatments III and IV) did not change the proportion of the diseased seedlings ($P=0.77$, $P=0.24$). Chemical control three days before inoculation in August resulted in a lower proportion of diseased seedlings as compared with other treatments I, III and IV ($P=0.034$, 0.054, 0.019, Tukey's test, Figure 4). The proportion of diseased seedlings in chemical control treatments made later (treatments III and IV) did not differ from the control (treatment I).

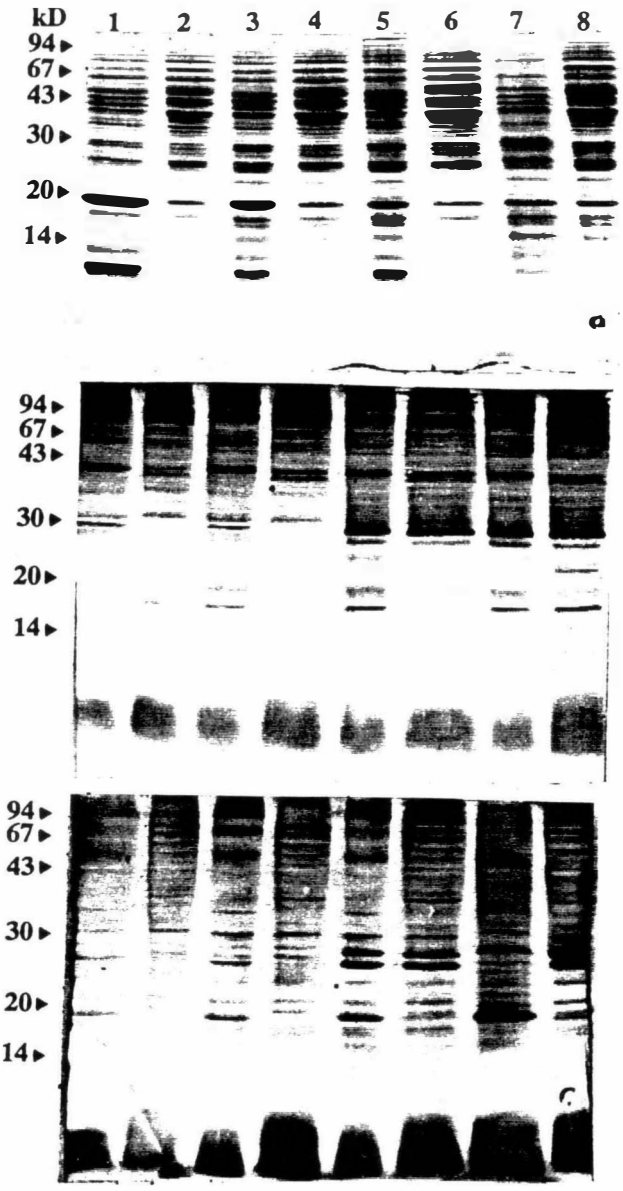

Figure 1. Protein pattern and immunoblot results of conidia and mycelia of isolates Ti2 (conidia lane 1; mycelia lane 2), Frh6 (lanes 3 and 4), Kemijarvi (lanes 5 and 6) and 9 (lanes 7 and 8).

a. The SDS-PAGE protein pattern by Coomassie staining.
b. Immunoblot using anti-conidia antibodies.
c. Immunoblot using anti-mycelia antibodies.

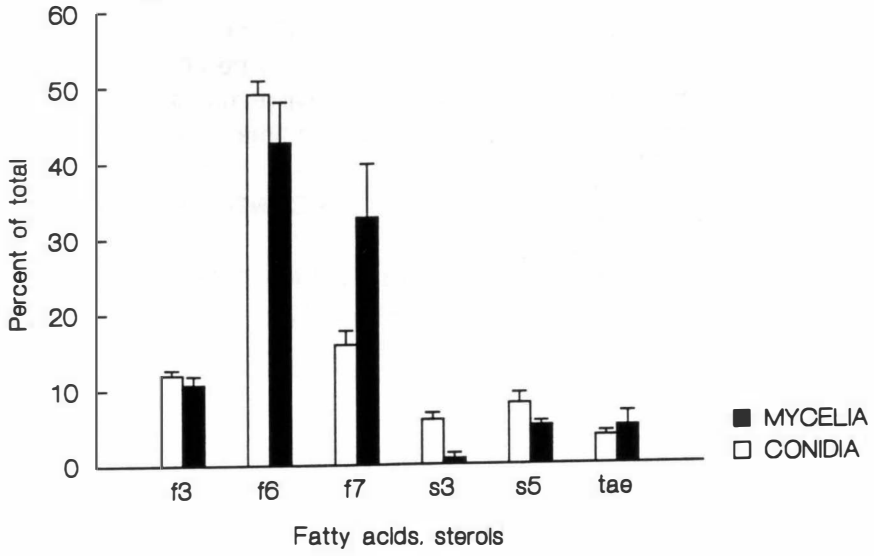

Figure 2. The proportion of fatty acid f3=hexadecanoic acid, f6=cis-9,12-octadecadienoic acid, f7=cis-9,12,15-octadecatrienoic acid and sterols s3=ergos-22-en-3-on and s5=ergosta-5,7,22-trien-3-ol of the total extracted fatty acid + sterols. Tae=the proportion of total amount of extracts from the sample dry weight. Samples were made from conidia and mycelia of five *G. abietina* isolates (Table 1). Bars indicate standard deviation.

DISCUSSION

Protein patterns of mycelial samples of *G. abietina* have been studied earlier and the two types (A and B) have been identified by immunoblot from mycelial samples (Petaisto et al. 1994, 1996). Although different media were used in the production of conidia, we found very little differences in protein patterns between conidia and mycelia. Some intensity differences were present, e.g., 20 kD band intensity was larger in conidia than in mycelia. Previously, the SDS-PAGE protein patterns of resting and swollen conidia of *Aspergillus fumigatus* differed from each others in number and intensity of bands, but the differences might have been caused by difficulty in extracting protein from resting conidia (Tronchin et al. 1995). However, proteins of conidia of *G. abietina* are easily extractable. Therefore, we feel our results indicated a true similarity between conidia and mycelia.

There were also many similarities in immunoblot results between conidia and mycelia. This indicated that immunological methods may be useful for diagnostics of this fungus. Some observed differences we found may be due to the different media used for production of conidia and mycelia. Extent of differences between mycelia and conidia that might be due to the different antigenicity of the development stages (conidia/mycelia) is unknown. However, the immunological differences found in mycelial samples between A-and B-type (Petaisto et al. 1996) can also be found also in conidial samples of these types. Some studies indicated that not all the structures of a fungus have the same antigens, e.g., conidia compared with mycelia in *Penicillium* (Cole et al. 1991) and yeast cell surface and surface of hyphae in *Candida albicans* (Deslaurier et al. 1996).

Fatty acids and sterols are important structural compounds in fungal membranes. Knowledge of fungal sterols and their biosynthesis is exploited in plant protection (Loeffler and Hayes 1992; Siegel 1981). Fatty acid and sterol analyses have been used as a taxonomic tool for *G. abietina* (Müller and Uotila 1997). Hexadecanoic acid (16:0), cis-9,12- octadecadienoic acid (9,12-18:2) and cis-9-octadecenoic acid (C18:1) are the principal fungal lipids (Losel 1988). The differences we found in proportions of C18:1 and in ergos-22-en-3-on and ergosta-5, 7,22-trien-3-ol between conidia and mycelia might indicate some functional and structural differences in membranes. Generally the lipids of spores and mycelia have been found to be rather similar (Weete 1984), but qualitative and quantitative differences in fatty acid composition are known between different structures of vesicular-arbuscular mycorrhizal fungi (Jabaji-Hare 1988).

Chlorothalonil is a nonsystemic foliar fungicide, effective against a wide spectrum of plant pathogens (Anonymous 1994). Effectiveness of the fungicide applied before inoculation and ineffectiveness performed three or 8-12 weeks after inoculation indicated that conidia were more susceptable to the fungicide than mycelia. Also, the fungus may have escaped the fungicide by growing into host tissues. Ylimartimo et al. (1995) noticed in their studies that three weeks after inoculation, the pathogen was observed on the host surface and in degenerating bract tissue. In SEM-observation from an earlier inoculation experiment, it was found that after 3 weeks, germtubes may be very short and even after 6 weeks germinating conidia may be restricted to host surfaces (Petaisto, unpublished). Therefore, chlorothalonil may be effective only at a very early stage of conidial germination. Further work is needed to identify optimal timing of fungicide treatments.

ACKNOWLEDGEMENTS

We thank Dr. M. M. Müller for analyzing the fatty acids and sterols.

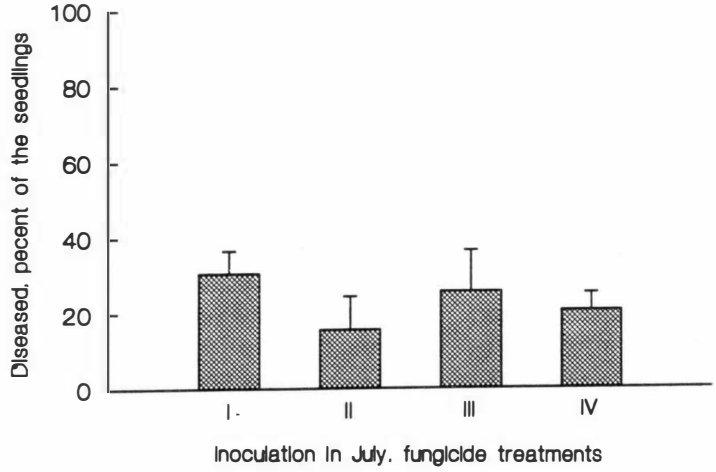

Figure 3. The percent of diseased seedlings in the spring following the experimental growing season. Inoculations were made in July. No chemical control=I, chemical control made 3 days before inoculation=II, 3 weeks=III or 12 weeks after inoculation=IV. Bars indicate standard deviation.

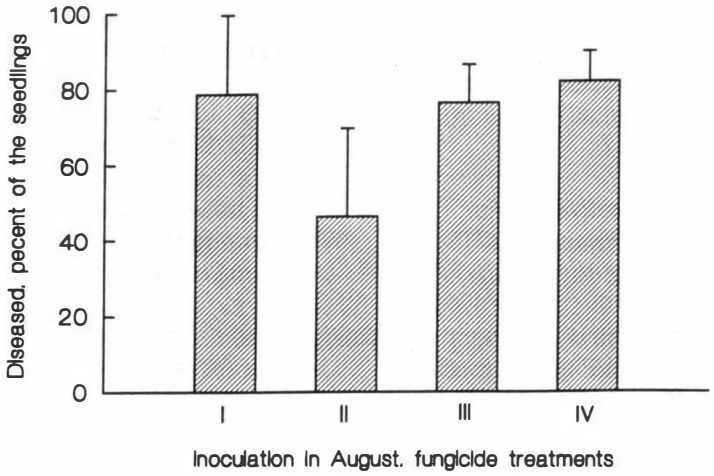

Figure 4. The percent of diseased seedlings in the spring following the experimental growing season. Inoculations were made in August. No chemical control=I, chemical control made 3 days before inoculation=II, 3 weeks=III or 8 weeks=IV after inoculation. Bars indicate standard deviation.

LITERATURE CITED

Akita, E.M. and S. Nakai. 1993. Comparison of four purification methods for the production of immunoglobulins from eggs laid by hens immunized with enterotoxigenic *E. coli* strain. Journal of Immunological Methods 160:207-214.

Anonymous. 1994. The Pesticide Manual. Tenth edition. The British Crop Protection council and The Royal Society of Chemistry. 1341 pp.

Cole, L., S. Hawes and F.M. Dewey. 1991. Immunocytochemical localization of specific antigen in cell walls of *Penicillium islandicum*. Mycological Research 95:1369-1374.

Deslaurier, N., J. Michaud, B. Carre and C. Leveille. 1996. Dynamic expression of cell-surface antigens probed with *Candida albicans*-specific monoclonal antibodies. Microbiology 14: 1239-1248.

Hamnede, M. 1980. Inokulering av tall med *Gremmeniella abietina*: en metod studie. Sveriges Lantbruksuniversitet, Instituti1n for skoglig genetik och vaxtfysiologi. International Report Number 15. 18 pp.

Hellgren, M. and N. Hogberg. 1995. Ecotypic variation of *Gremmeniella abietina* in northern Europe - disease patterns reflected by DNA variation. Canadian Journal of Botany 73:1531-1539.

Jabaji-Hare, S. 1988. Lipid and fatty acid profiles of some vesicular- arbuscular mycorrhizal fungi: contribution to taxonomy. Mycologia 80:622-629.

Kokko, H., I. Kur1n and S. Karenlampi. 1994. Rapid production of antibodies in chicken and isolation from eggs. *In*: Cells, J. E. (ed.). Cell Biology: A Laboratory Handbook, Vol 2. Academic Press, San Diego. pp. 282-288.

Koski, V. and R. Sievanen. 1985. Timing of growth cessation in relation to variations in the growing season. *In*: Tigerstedt, P.M.A., P. Putt1n and V. Koski (eds.). Crop physiology of forest trees. Helsinki University Press, Helsinki, Finland. pp. 167-169.

Loeffler, T.R.S. and A.L. Hayes. 1992. Effects of sterol biosynthesis inhibitor fungicides on growth and sterol composition of *Ustilago maydis*, *Botrytis cinerea* and *Pyrenophora teres*. Pesticide Science 36:7-17.

Losel, D.M. 1988. Fungal lipids. *In*: C. Ratlege and S.G. Wilkinson (eds). Microbial Lipids. Academic Press, London and New York. pp. 699-806.

Müller, M.M., R. Kantola and V. Kitunen. 1994. Combining sterol and fatty acid profiles for characterization of fungi. Mycological Research 98:593-603.

Müller, M.M. and A. Uotila. 1997. The diversity of *Gremmeniella abietina* var. *abietina* FAST-profiles. Mycological Research (In press).

Parviainen, J. and L. Tervo. 1989. A new approach for production of containerised coniferous seedlings using peat sheet coupled with root pruning. Forestry Supplement 62:87-94.

Petaisto, R L. and T. Kurkela. 1993. The susceptibility of Scots pine seedlings *Gremmeniella abietina*: effect of growth phase, cold and drought stress. European Journal of Forest Pathology 23:385-399.

Petaisto, R. L., T.E. Rissanen, R.J. Harvima and E.O. Kajander. 1994. Analysis of the protein pattern of *Gremmeniella abietina* with special reference to protease activity. Mycologia 86:242-249.

Petaisto, R. L., A. Uotila, M. Hellgren, J. Kaitera, J. Tuomainen and E.O. Kajander. 1996. Two types of the European race of *Gremmeniella abietina* can be identified with immunoblotting. Mycologia 88:619-625.

Siegel, M.R. 1981. Sterol-inhibiting fungicides: effects on sterol biosynthesis and sites of action. Plant Disease 65:986-989.

Systat for windows 1992. Statistics, Version 5 edition. I.L. Evanston (ed.). Systat Inc. 750 pp.

Tronchin, G., J.P. Bouchara, M. Ferron, G. Larcher and D. Chabasse. 1995. Cell surface properties of *Aspergillus fumigatus* conidia: correlation between adherence, agglutination, and rearrangements of the cell wall. Canadian Journal of Microbiology 41:714-721.

Uotila, A. 1983. [Physiological and morphological variation among Finnish *Gremmeniella abietina* isolates]. Communications of the Finnish Forest Research Institute 119:1-12.

Weete, J.D. 1980. Lipid Biochemistry of Fungi and Other Organisms. Plenum Press, New York. 388 pp.

Ylimartimo, A., G. Laflamme, M. Simard and D. Rioux. 1995. Ultrastructural and cytochemical results on scleroderris canker infection of red pine seedlings. Pp. 146-153. *In*: Capretti, P., U. Heiniger and R. Stephan (eds.). Shoot and Foliage Diseases in Forest Trees. Proceedings of the IUFRO Working Parties S2.06.02 and S2.06.04. Vallobrosa, Firenze June 6-11, 1994. pp. 146-153.

PATHOGENICITY AND GENETIC VARIATION IN PHYTOPHTHORA CACTORUM[1]

A. Lilja[2], J. Hantula[2], R. Karjalainen[3], K. Kammiovirta[3]
and H. Nuorteva[2]

ABSTRACT

Inoculations of wounds with *Phytophthora cactorum* isolates from stem lesions on *Betula pendula* seedlings or from *Fragaria* x *ananassa* plants suffering from nrown rot resulted in necrotic lesions on silver birch. Most of 2-month-old seedlings inoculated in spring with *P. cactorum* isolates from birch or strawberry died or broke during the same summer. Lesion size on 1-year-old silver birch seedlings caused by isolates from birch were large compared to those caused by an isolate from strawberry or other fungi occurring in stem lesions. Only isolates from birch caused clear lesions on intact bark. DNA-analyses, random amplified microsatellites (RAMS) and random amplified polymorphic DNA (RAPD) revealed variation within *P. cactorum* isolates; both analyses separated the isolates from silver birch and strawberry plants. These two groups seem to be separate populations and isolates from strawberry may be clonal.

INTRODUCTION

Different diseases of many plants have been attributed to *Phytophthora cactorum* (Leb. and Cohn) Schr. Strawberry crown rot was first reported in 1952 from Germany (Deutschmann 1954), but has since been reported in many other European countries. In Finland *P. cactorum* has caused crop losses in strawberry (*Fragaria* x ananassa Duch.) fields since 1990 (Parikka 1990). A year later it was isolated for the first time from necrotic stem lesions on silver birch (*Betula pendula* Roth) seedlings growing in foressst nurseries (Lilja and Hietala 1994). In later studies, the percentage of *P. cactorum* in diseased seedlings varied from 20-80 in different nurseries (Lilja et al. 1996b). Other fungi isolated from birch stem lesions included *Godronia multispora* J.W. Groves, *Fusarium avenaceum* (Corda ex Fr.) Sacc, *Alternaria alternata* (Fr.) Keissl., *Botrytis cinerea* Pers. ex Nocca & Balb., and *Phomopsis* sp. (Kurkela 1974; Lilja and Hietala 1994; Lilja et al. 1996b; Petaisto 1983).

Many of the morphological characters used in *Phytophthora* systematics, such as sporangial and oogonial dimensions may exhibit high levels of variation within a species and may overlap between species (Stamps et al. 1990; Waterhouse 1963). In spite of possible morphological variation, first genetic analyses based on isozymes (Oudemans and Coffey 1991) and mitochondrial DNA (Foster and Coffey 1991) suggested that *P. cactorum* isolates from diverse geographical locations and host plants share a high degree of similarity.

In this study, we investigated the similarity of *P. cactorum* isolates originating from stem lesions on silver birch and from strawberry plants suffering from crown rot by pathogenicity tests and DNA markers.

MATERIALS AND METHODS

Phytophthora isolates:

Finnish *P. cactorum* isolates originated from stem lesions on nursery-grown silver birch seedlings or from strawberry plants suffering from crown rot. Several *P. cactorum* isolates from strawberry in Europe were included as reference isolates in DNA analyses. The Finnish isolates were from 10 forest nurseries and eight strawberry fields located in different parts of Finland. More detailed information on the isolates is given in Table 1.

Pathogenicity tests:

The pathogenicity of *P. cactorum* to birch was tested in two inoculation experiments. Four test isolates were from silver birch (Ph10, Ph11, Ph18 and Ph20) and two from strawberry (S3 and S13). To compare pathogenicity with other fungal species isolated

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Table 1. Sources of *Phytophthora cactorum* isolates used for pathogenicity and genetic variability evaluations.

Isolate Designation	Host	County of Origin	Source
S3, S5, S6, S7, S9, S10, S13, S14, S15, S19	*Fragaria* x *ananassa*	Finland	P. Parikka
145 (H)	*Fragaria* x *ananassa*	England	D. Harris
S21 (Ph17)	*Fragaria* x *ananassa*	Finland	J. Tegel
CH09, CH12, CH15, CH19	*Fragaria* x *ananassa*	Sweden	C. Olsson
CH17	*Fragaria* x *ananassa*	Estonia	C. Olsson
A1, W1	*Fragaria* x *ananassa*	Germany	S. Werres
2/94-III	*Aesculus hippocastanum*	Germany	S. Werres
1557, 9/88/92	*Rhododendron* sp.	Germany	S. Werres
5/94	*Faqus sylvatica*	Germany	S. Werres
Ph2, Ph3, Ph4, Ph5, Ph8, Ph10, Ph11, Ph14, Ph15, Ph18, Ph20, Ph21, Ph22, Ph23, Ph24	*Betula pendula*	Finland	A. Lilja

from stem lesions, inoculations were also done with *B. cinerea*, *F. avenaceum*, *Godronia* sp., *Phomopsis* sp. and binucleate *Rhizoctonia* sp. Experiments were conducted in a greenhouse.

Experiment with 2-month-old silver birch seedlings:

Silver birch seedlings were produced according to standard nursery practice in a greenhouse. In May, 400 seedlings were transplanted into pots (0.5 L), two seedlings per pot. Growing media consisted of fertilized, low-humified Sphagnum peat (Finn peat M6, Kekkila Corpl, Finland). Two weeks after transplantation forty, 2-month-old, non-wounded seedlings were inoculated with each of four *P. cactorum* isolates (Ph18, Ph20, S3 and S13) or with pure agar blocks (control). Wounding occurred just before inoculation by removing a 3mm x 3mm piece of periderm with a scalpel at a height of 5cm above the root collar. Inoculum consisted of a 3mm x 3mm agar block from a 1-week-old *P. cactorum* culture on potato dextrose agar (PDA) or a pure, uninoculated PDA block. Inoculum was placed on the wound or on sound bark and secured with parafilm. Ten pots representing the same treatment were placed on a tray. Each tray served as a replication and each treatment had four replications. Trays were arranged in the greenhouse in a randomized design. Seedlings were watered 3-4 times a week carefully to avoid splashes. Seedling conditions were evaluated every second week; seedlings were divided into five categories according to the spread of fungal infection: 1) no lesion or lesion $<9mm^2$, 2) lesion $>9mm^2$, but not over half the stem diameter, 3) lesion over half the stem diameter, 4) lesion around the stem but seedling alive (stem broken), and 5) lesion around the stem, seedling dead (stem may or may not be broken). The final examination was done 3 months after inoculation. The disease index of seedlings was calculated from the frequency (f) of different condition categories (c1-c5):(fc1+2fc2 +3fc3+4fc4+5fc5)/N. The data were analyzed using Kruskal-Wallis analysis and a nonparametric test for treatment comparisons.

Experiment with 1-year-old silver birch seedlings:

Eighty 1-year-old, nursery-grown silver birch seedlings were taken in spring from cold storage (-4°C). After thawing, they were transplanted into pots (5 l)

containing the same growing media as described above. Two weeks after transplanting, 20 unwounded and 20 wounded seedlings were inoculated with each of 3 *P. cactorum* isolates (Ph10, Ph11 and S3)(Table 1), the other test fungi, or pure PDA blocks (controls). Wounding and inoculations were conducted as described above. Ten seedlings represented each treatment and the plants were arranged within the greenhouse in a randomized design. Condition of all seedlings was monitored 3 months after inoculation. Stem lesion margins were drawn with lumocolor pencils on overhead film (PPC, Cannon) and the size of lesions were measured with an image analyzer Leica Q500MC of the drawings. The data were analyzed using one- and two-way analyses of variance and the mean differences determined using Duncan's multiple-range test.

DNA analyses:

DNA extractions:

DNA was isolated as described by Hantula et al. (1996). The protocol included cell disruption, two phenol:chloroform (1:1) extractions, a chloroform: isoamyl alcohol (24:1) extraction, and precipitation with alcohol follwed by drying under vacuum. DNA was resuspended in 10mM Tris-HCl buffer (pH 8.0) containing 1mM EDTA.

Random amplified microsatellite (RAMS) analysis:

The PCR-reactions were carried out in reaction conditions suggested by the manufacturer of the Dynazyme II DNA-polymerase (Finnzymes Ltd., Finland), except the concentration of the primar was 2uM. Samples were denatured by 10 minute incubation at 95°C after which 35 (CGA- and GT-primers) or 37 (CCA-primer) cycles of amplification were carried out as described by Hantula et al. (1996). The amplification products were separated by electrophoresis in gels containing 0.8% SynerGel (Diversified Biotech) and 0.8% agarose (FMC BioProducts) run in TAE-buffer (40mM Tris-Acetate pH 8.0, 1 mM EDTA). Differences between *P. cactorum* isolates were calculated by scoring the number of differences in 19 RAMS markers. From this data, we constructed a similarity matrix according to the formula:

$$S = 2 \times nab/(na + nb),$$

where na and nb represent the total number of bands present in lanes a and b, respectively, and nab is the number of bands which are shared by both lanes. The matrix was used to create UPGMA dendrograms using the MEGA software (Kumar et al. 1993).

Random amplified polymorphic DNA (RAPD) analysis:

Amplification was performed as described by Lilja et al. (1996a) except the polymerase buffer (200mM (NH4)2SO4, 750 mM Tris-HCl, pH 9.0, 1% (w/v) Tween) and polymerase (0.6U of Red Hot polymerase, Advanced Biotechnologies, Ltd., UK) were different. Amplification products were electrophoresed in 1.4% agarose gels with 1 x TAE (40mM Tris-Acetate, pH 8.0, 1mM EDTA) and stained with ethidium bromide. Amplifications were repeated twice for each isolate and only reproducible bands were scored. The primers were selected from Operon Kit B (Operon Technologies Inc., Alameda, CA, USA). All twenty primers were tested, and the two best ones (OPB-11: GTAGACCCGT and OPB-18: CCACAGCAGT) were selected for further study. The statistical analysis of RAPD data, dissimilarity coefficients (DIST) and clustering analysis were based on the NTSYS program (Rohlf 1992).

RESULTS

Pathogenicity tests:

Inoculation of wounds and intact bark on birch stems with *P. cactorum* isolates from birch resulted in necrotic lesions identical to those occurring naturally in forest nuseries within 2 weeks. Isolates from strawberry caused necrotic lesions only in wounded seedlings (Figures 1 and 3).

Percentage of dead and broken 2-month-old seedlings varied at the end of the experiment depending on isolate tested and whether wounding occurred prior to inoculation. Wounding seedlings prior to inoculation with *P. cactorum* isolates resulted in large lesions; percentages of broken and dead birch seedlings inoculated with isolates from birch and strawberry plants were more than 10 and 70% , respectively (Figure 1). In comparison, the percentage of broken and dead seedlings resulting from inoculations of non-wounded stems with birch isolates was 13 (Figure 2). Lesions caused by inoculations of non-wounded seedlings with isolates from strawberry were small; only 7.5% of inoculated 2-month-old sliver birch seedlings were affected (Figure 2).

On 1-year-old seedlings, the size of lesions caused by *Godronia* sp., *B. cinerea*,binucleate *Rhizoctonia*

sp., *Phomopsis* sp. and the *P. cactorum* isolate from strawberry were small compared to those caused by *P. cactorum* isolates from birch ($P<0.01$). Lesions caused by *F. avenaceum* and *Phomopsis* sp. were similar to those caused by the *P. cactorum* isolate from strawberry (Figure 3).

Variation among P. cactorum isolates:

Both RAMS and RAPD analyses revealed variation among *P. cactorum* isolates; banding patterns of isolates from strawberry were different than those from birch. There were few minor differences in UPGMA dendrograms depending on the method used (Figures 4 and 5). However, apparently *P. cactorum* isolates from birch and strawberry form two separate groups. Also, there was no variation within isolates from strawberry, suggesting that isolates from strawberry may be clonal.

DISCUSSION

This study suggested that *P. cactorum* infecting birch and strawberry are pathogenically and genetically separate. Isolates from birch were highly pathogenic to their host plant and only these isolates caused necrotic lesions on non-wounded bark. Isolates from strawberry were capable of infecting birch through wounds. Both RAMS and RAPD analyses separated silver birch from strawberry isolates.

Several fungi isolated from stem lesions in Finnish forest nurseries are thought to be pathogenic to birch. For example, *F. avenaceum* has been isolated from necrotic tissue around wounds made by *Cicadella viridis* (L.) for oviposition (Juutinen et al. 1076) and densely-grown seedlings are readily attacked by *B. cinerea* (Lilja 1980). *G. multispora* has commonly been isolated from diseased silver birch in some nurseries (Petaisto 1983; Rikala and Petaisto 1986) and also from cankers in young birch stands growing on peat and paludified mineral soil (Kurkela 1974). In Canada, a *Phomopsis* sp. was found on dying tops of yellow birch (*B. alleghaniensis* Britton)(Horner 1956). Arnold (1970) expressed the view that this fungus [teleomorph: *Diaporthe alleghaniensis* R. H. Arnold (Arnold 1967)], could be a serious pathogen of stressed seedlings. Because our *Godronia* sp. and *Phomopsis* sp. isolates did not form a perfect state, it was not possible to identify them to species.

Apparently, only *P. cactorum* isolates from strawberry can cause crown rot in strawberry (Kennedy and Duncan 1995; Seemuller and Schmidle 1979; van der Scheer 1971). The high genetic similarity of *P. cactorum* isolates from strawberries suffering from crown rot in studies by Cooke et al. (1996) supports our findings and the conclusion of a distinct strawberry crown rot pathotype.

Most new strawberry plants are produced vegetatively. Contaminated runner plants may serve distribution of a single *P. cactorum* clone. The influence of pathoen migration on reducing genetic variability has been previously found in a *Phytophthora infestans* (Mont.) de Bary population (Goodwin et al. 1994) where contaminated seed tubers were an important source of pathogen spread. Therefore, we conclude that it is possible that crown rot in Europe is caused by a single clone of *P. cactorum*.

More than 60 years ago, Leonian (1934) concluded that *P. cactorum* was one of the "good" species of *Phytophthora*, i. e., it could be identified by key morphological characteristics. Oudemans and Coffey (1991) could separate morphologically different *P. cactorum* isolates using isozyme analysis and they recognized two electrophoretic *cactorum* groups. Two of their isolates represented CACT2-group and were isolated from strawberry in New York. Other tested isolates belonged to CACT1 and they originated from diverse geographical locations and host plants. In a later study, RAPD analysis revealed two subspecific groups within *P. cactorum* isolates from apple, strawberry and raspberry; tested raspberry isolates had affinities with both strawberry and apple isolates (Cooke et al. 1996). We conclude from both pathogenicity tests and DNA analyses reported here and from other studies that *P. cactorum* has evolved into genetically distinct populations on different hosts.

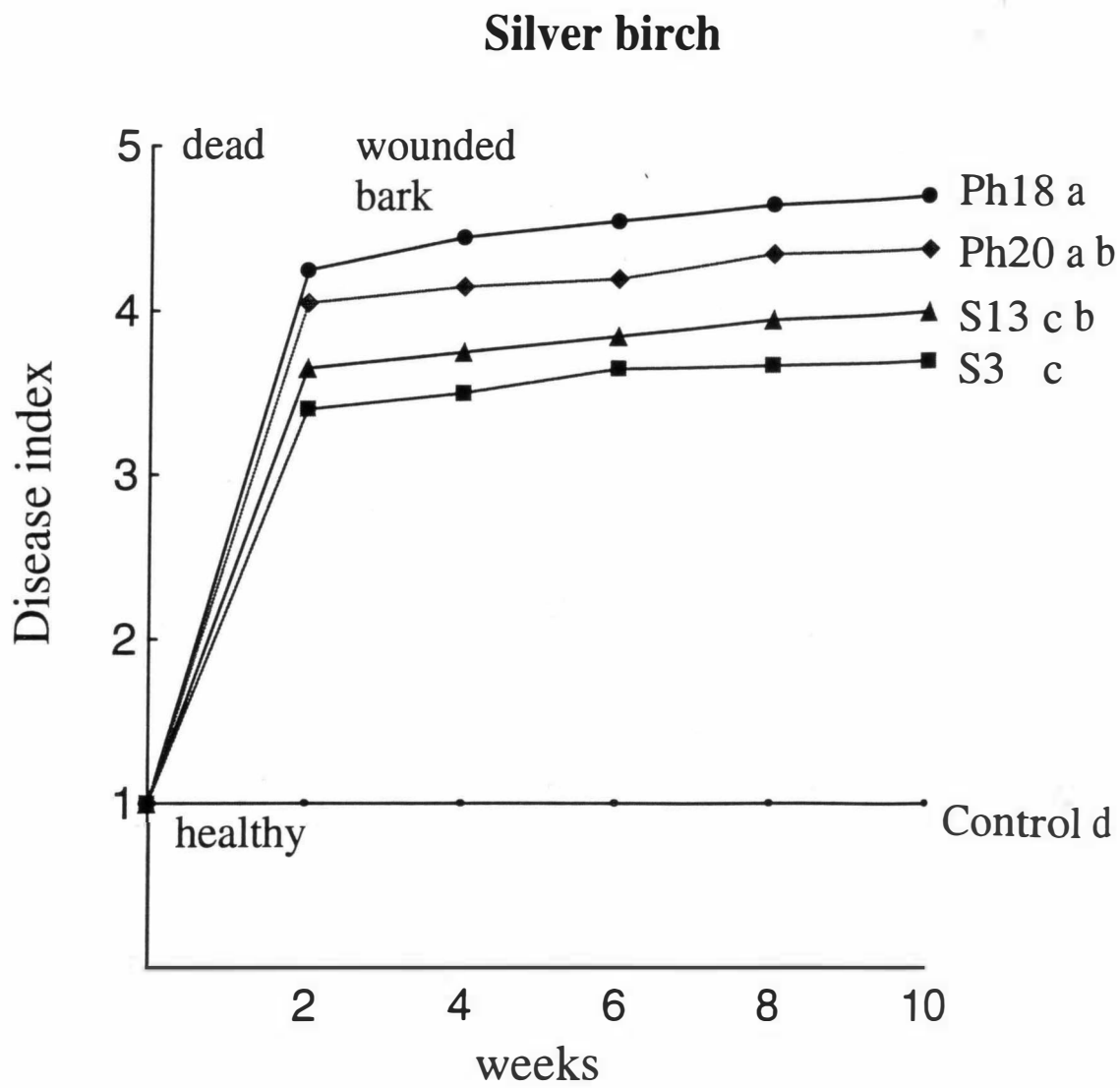

Figure 1. Condition of young silver birch seedlings 10 weeks after inoculation with *Phytophthora cactorum* isolates from stem lesions on silver birch (Ph18 and Ph20) and strawberry plants suffering from crown rot (S3 and S13). Inoculations were done on wounded bark of 2-month-old seedlings. Curves followed by the same letter indicate that the health condition of seedlings does not differ ($P > 0.01$).

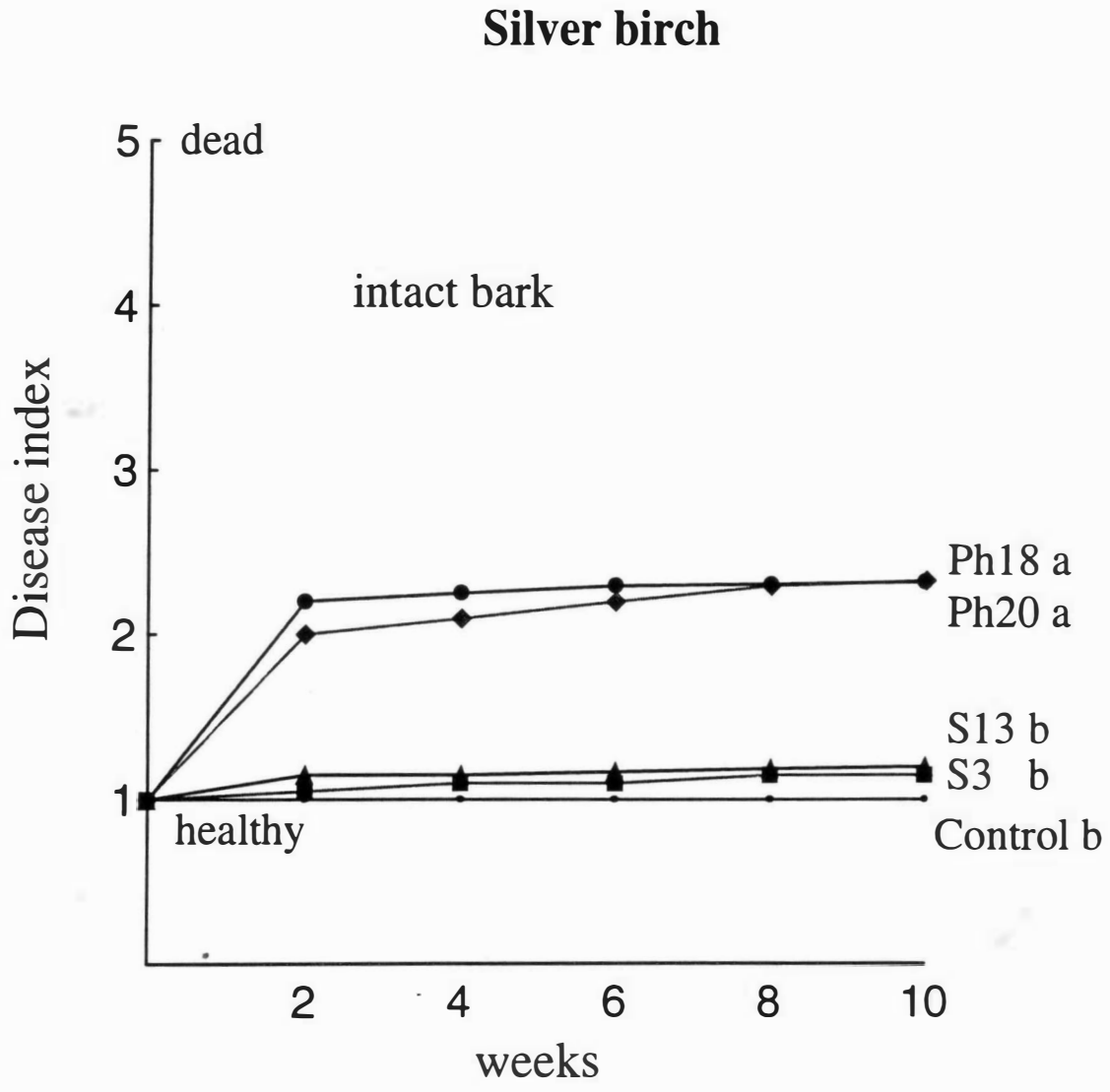

Figure 2. Condition of young birch seedlings 10 weeks after inoculation with *Phytophthora cactorum* isolates from stem lesions on silver birch (Ph18 and Ph20) and strawberry plants suffering from crown rot (Se and S13). Inoculations were done on intact bark of 2-month-old seedlings. Curves followed by the same letter indicate that the health condition of seedlings does not differ ($P>0.01$).

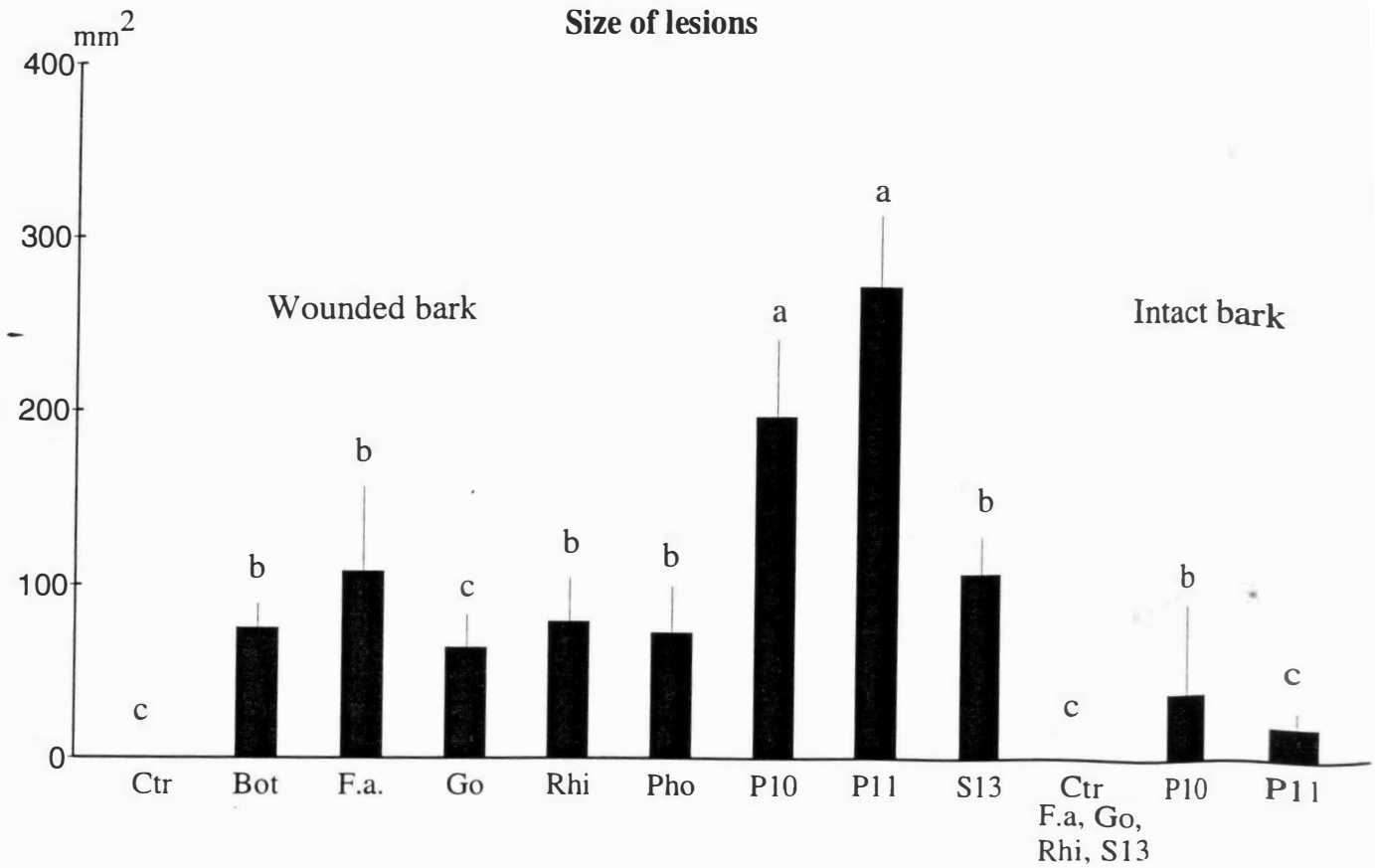

Figure 3. The size of lesions on silver birch seedlings three months after inoculation with *Botrytis cinerea* (Bot), *Fusarium avenaceam* (F.a.), *Godronia* sp. (Go), binucleate *Rhizoctonia* sp. (Rhi), *Phomopsis* sp. (Pho) and *Phytophthora cactorum* isolates (Ph10 and Ph11) from stem lesions on silver birch and *P. cactorum* (S13) from strawberry plants suffering from crown rot. Inoculations were done on wounded bark of 1-year-old seedlings. The same letter above columns indicates that the size of lesions does not differ ($P > 0.01$). Bars represent standard errors.

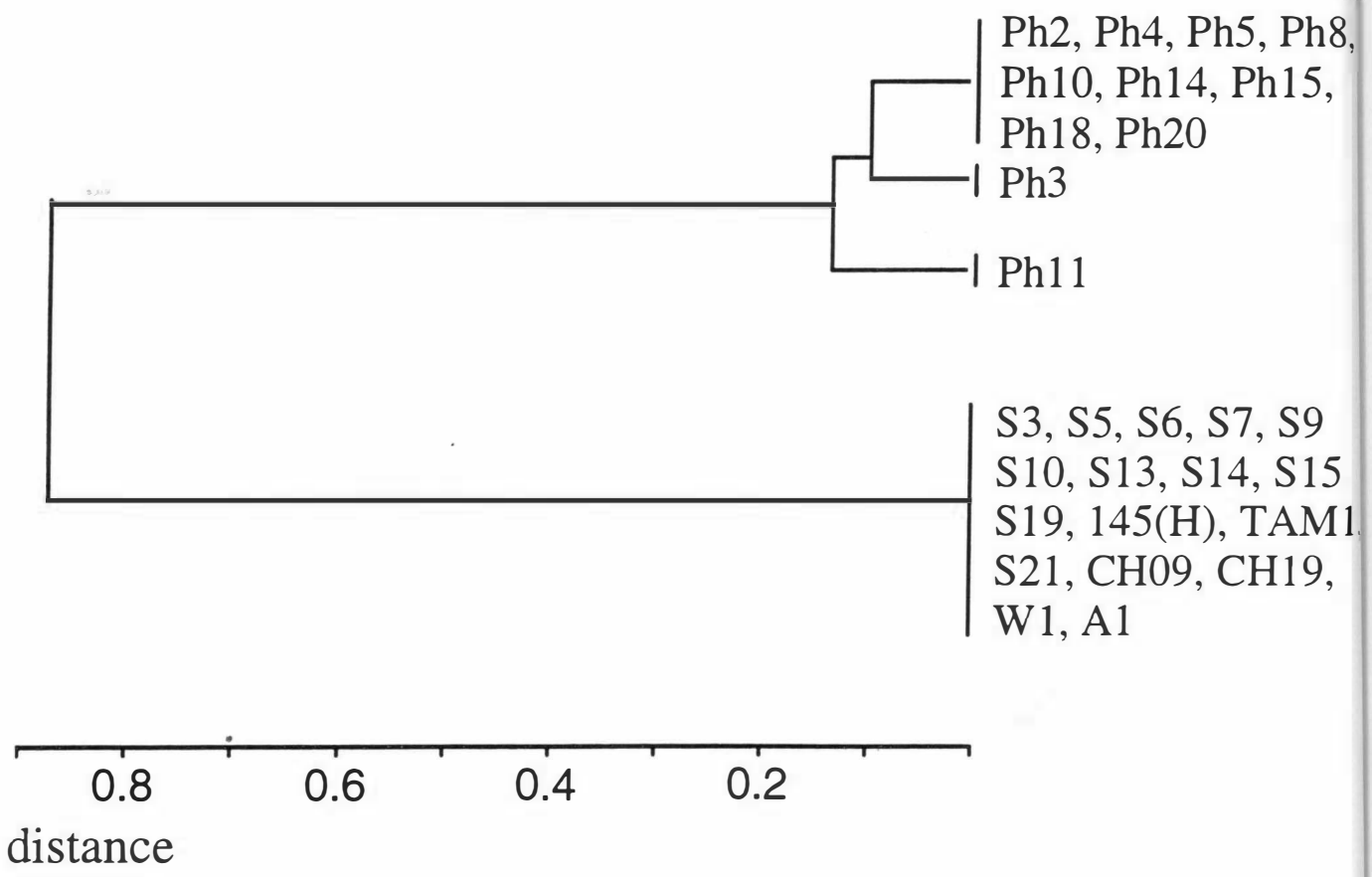

Figure 4. An UPGMA dendrogram of relationships among *Phytophthora cactorum* isolates from silver birch (Ph) and strawberry (all other isolates). The genetic distance given by RAMS-markers.

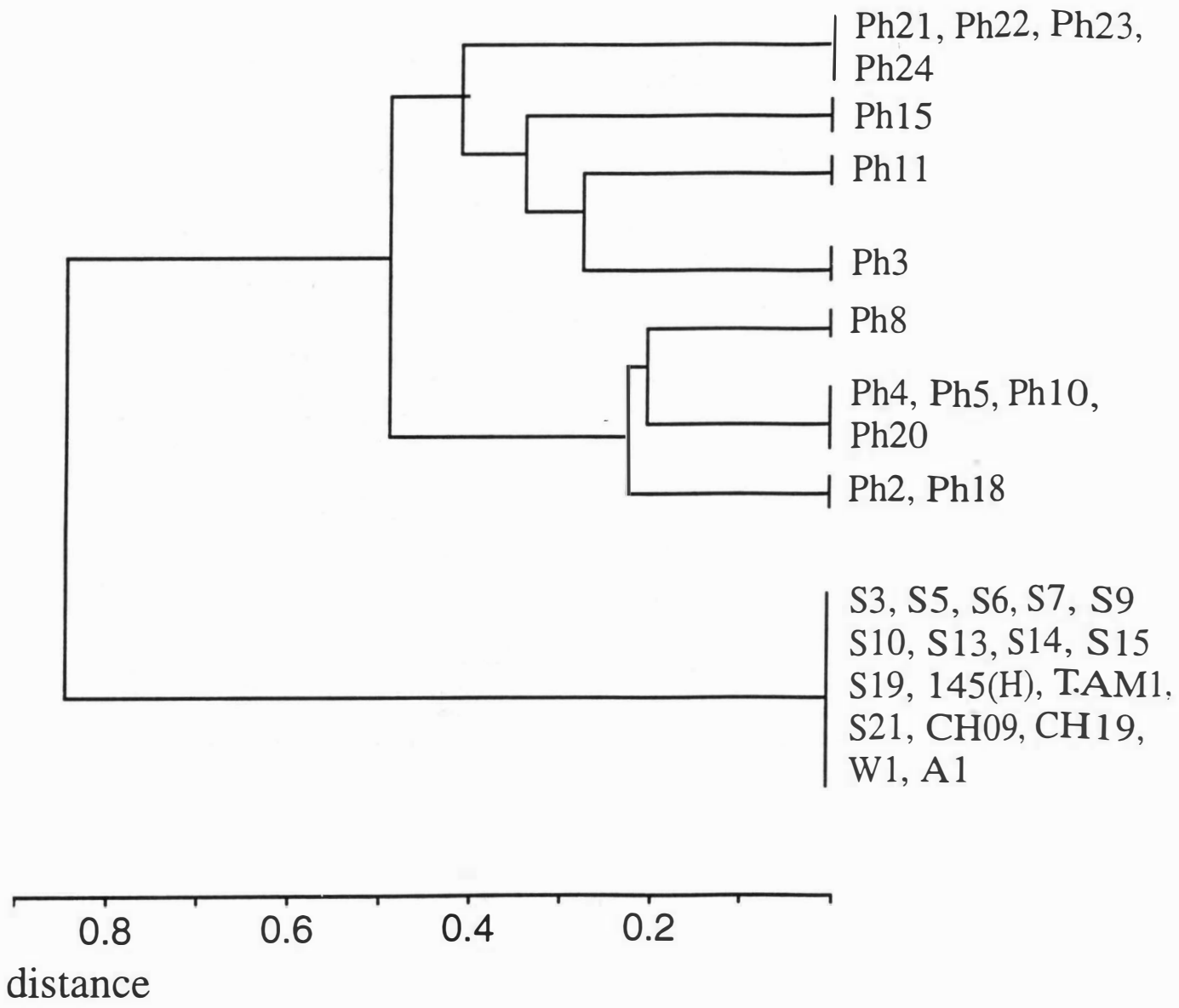

Figure 5. An UPGMA dendrogram of relationships among *Phytophthora cactorum* isolates from silver birch (Ph) and strawberry (all other isolates). The genetic distance given by RAPD-markers.

LITERATURE CITED

Arnold, R.H. 1967. A canker and foliage disease of yellow birch. I. Description of the casual fungus, *Diaporthe alleghaniensis* sp. nov., and the symptoms on the host. Canadian Journal of Botany 45:783-801.

Arnold, R.H. 1970. A canker and foliage disease of yellow birch. II. Artificial infection studies with *Diaporthe alleghaniensis*. Canadian Journal of Botany 48:1525-1540.

Cooke, D.E.I., D.M. Kennedy, D.C. Guy, J. Russell, S.E. Unkles and J.M. Duncan. 1996. Relatedness of Group I species of *Phytophthora* as assessed by randomly amplified polymorphic DNA (RAPDs) and sequences of ribosomal DNA. Mycological Research 100:297-303.

Deutshmann, F. 1954. Eine Wurzelfaule an Erdbeeren, hervorgerufen durch *Phytophthora cactorum* (Leb. et Cohn) Schroet. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 6:7-9.

Foster, H. and M.D. Coffey. 1991. Approaches to the taxonomy of *Phytophthora* using polymorphisms in mitochondrial and nuclear DNA. *In*: Lucas, J. A., R. C. Shattock, D. S. Shaw and L. R. Cooke (eds.). *Phytophthora*. Cambridge University Press, Cambridge, UK. pp. 164-183.

Goodwin, S.D., B.A. Cohen and W.E. Fry. 1994. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. Proceedings of the National Academy of Sciences (USA) 91:11591-11595.

Hantula, J., M. Dusabenyagasani and R.C. Hamelin. 1996. Random amplified microsatellites (RAMS) - a novel method for characterizing genetic variation within fungi. European Journal of Forest Pathology 26:159-166.

Horner, R.M. 1956. *Diaporthe* canker on *Betula utea*. *In*: Proceedings of the 22nd Session of the Canadian Phyopathological Society, Edmonton 1955, 23:16-17.

Juutinen, P., T. Kurkela and S. Liilja. 1976. [*Cicadella viridis* (L.), as a wounder of hardwood saplings and infections of wounds by pathogenic fungi]. Folia Forestalia 284:1-12. (in Finnish).

Kennedy, D.M. and J.M. Duncan. 1995. A papillate *Phytophthora* species with specificity to *Rubus*. Mycological Research 99:57-68.

Kumar, S., K. Tamura and M. Nei. 1993. MEGA:Molecular Evolutionary Genetics Analysis. Version 1.01. The Pennsylvania State University, University Park, PA.

Kurkela, T. 1974. *Godronia multispora* Groves (Helotiales) and its pathogenicity to *Betula verrucosa* Ehr. and *B. pubescens* Ehr. Karstenia 14:33-45.

Leonian, L.H. 1934. Identification of *Phytophthora* species. West Virginia Agricultural Experiment Station Bulletin 262:1-36.

Lilja, A. 1980. Taimitarhan tarkeimmat sienituhot. Metsanviljelyn koeaseman tiedonantoja 9:21-26.

Lilja, A. and A. Hietala. 1994. *Phytophthora cactorum* and a novel type *Rhizoctonia* sp as forest nursery pathogens. *In*: Perrin, R. and J. R. Sutherland (eds.). Proceedings of the second meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Les Colloques No. 68. INRA, Paris. pp. 59-64.

Lilja, A., A. Hietala and R. Karjalainen. 1996. Identification of a uninucleate *Rhizoctonia* sp. by pathogenicity, hyphal anastomosis and RAPD analysis. Plant Pathology 45:997-1006.

Lilja, A., R. Rikala, A. Hietala and R. Heinonen. 1996. Stem lesions on *Betula pendula* seedlings in Finnish forest nurseries and the pathogenicity of *Phytophthora cactorum*. European Journal of Forest Pathology 26:89-96.

Oudemans, P. and M.D. Coffey. 1991. Isozyme comparison within and among worldwide sources of three morphologically distinct species of *Phytophthora*. Mycological Research 95:19-30.

Parikka, P. 1990. Nahkamata vaivaa mansikkaa. Puutarha 9/90, 630-631.

Petaisto, R.-L. 1983. [Stem spotting of birch (*Betula pendula*) in nuseries]. Folia Forestalia 544:1-9. (in Finnish).

Rikala, R. and R.-L. Petaisto. 1986. [Effects of fertilization on the nutrient concentration, growth and incidence of stem spotting in bare-rooted birch transplants]. Folia Forestalia 642:1-9. (in Finnish).

Rohlf, F.J. 1992. NTYSYS-pc. Numerical taxonomy and multivariate analysis system. Exeter Publishing, Ltd., Setauket, NY.

Seemuller, E. and A. Schmidle. 1979. EinfluB der Herkunft von *Phytophthora cactorum*-Isolaten auf ihre Virulenz an Apfelrind, Erdbeerrhizomen und Erbeerfruchten. Phytopathologische Zeitschrift 94:218-225.

Stamps, D.J., G.M. Waterhouse, F.J. Newhook and G.S. Hall. 1990. Revised key to the species of *Phytophthora*. Mycological Papers (Commonwealth Mycological Institute) 162. 28p.

van der Scheer, H.A.T.H. 1971. Isolation of *Phytophthora cactorum* from soil orchards and strawberry fields and differences in pathogenicity to apple. Netherlands Journal of Plant Pathology 77:65-72.

Waterhouse, G.M. 1963. Key to species of *Phytophthora* deBary. Mycological Papers (Commonwealth Mycological Institute) 92. 22p.

THE IMPORTANCE OF MYCORRHIZAL INOCULATION IN FOREST NURSERIES[1]

N. Fonseca[2]

ABSTRACT

Inoculation procedures to make controlled mycorrhization a common nursery practice in Portugal are described. Inoculating nursery seedlings with specific fungi produces high-quality stock with good performance following outplanting in the field. Symbiotic fungal species associated with forest trees were collected during field surveys. It was found that in stands where symbionts occurred, some diseases were not detected. *In vitro* tests confirmed antagonism between symbiotic fungi and some forest pathogens. Special emphasis was placed on using edible mushrooms as symbionts for mycorrhization because of their potential economic benefit.

INTRODUCTION

Mycorrhizae in nurseries have been an important area of research because of their potential for providing high-quality seedlings for outplanting in the field. Benefits of mycorrhizae, fungal-tree root symbiotic relationships, are enormous for several reasons. Mycorrhizae increase uptake of nutrients such as phosphorus and nitrogen by converting the nutrients to more assimilable forms for use by trees. In addition, root surface area is increased by the external fungal hyphae accessing nutrients and water that may be out of reach of normal tree roots. Thus, mycorrhizae may provide drought resistance to seedlings. Mycorrhizae may also be important in disease protection. The mycorrhizal mantle functions as a mechanical barrier against root pathogens; carbohydrates released from root tips are utilized by mycorrhizal fungi, thus preventing soil pathogens from using this important food source. Symbiotic fungi may also be antagonistic to root pathogens; antibiotics active against soilborne fungi may be produced by the symbiont and a microflora antagonistic toward soil pathogens is often favored by mycorrhizal symbionts (Azevedo 1973; Fonseca and Azevedo 1990, 1992, 1994; Zak 1964). As a result of these benefits, outplanting survival and growth of mycorrhizal seedlings are often superior to non-mycorrhizal seedlings.

Field surveys in Portugal indicated that on sites where mycorrhizal symbionts were collected, occurrence of root disease caused by *Heterobasidion annosum* (Fr.) Bref. was not detected. On the other hand, in stands where these symbionts were absent or rare, *H. annosum* was found attacking trees (Azevedo 1973; Fonseca and Azevedo 1994). Azevedo (1973) studied *in vitro* anatagonism of several mycorrhizal symbionts against *H. annosum*. Stong inhibition was observed by *Lactarius deliciosus* Fr. against the pathogen. Lower levels of inhibition were detected with *Boletus* (= *Suillus*) *luteus* Lin.:Fr., *B.* (= *Ixocomus*) *granulatus* L:Fr. and *B.* (= *Suillus*) *bovinus* (L.:Fr.) Qu.

In addition to the advantages of mycorrhizal fungi to tree performance, several important symbionts have been identified as important edible fungi. Interest in producing and gathering edible fungi in forest environments is increasing worldwide, especially in Portugal. Because of the importance of mycorrhizal fungi in forests, procedures were developed to routinely inoculate nursery seedlings with specific mycorrhizal symbionts.

INOCULATION PROCEDURES

In the past, production of seedlings with ectomycorrhizae involved inoculating with pure fungal cultures in peat-vermiculite growing media and using inoculated compost as a supplement to growing media. Although these procedures produced satisfactory results, they required extensive effort and were expensive. To overcome these limitations, another method was developed which incorporated desired symbionts in a dry powder. Such a powder has the advantage of easy handling and reduced volume and weight compared to other inocula.

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Specific fungal fruiting bodies were collected during surveys for several years. The fruiting bodies were carefully cleaned and then dried at 25°C. After drying, sporophores were ground to a fine powder and stored in polyethylene bags under dry conditions until needed. Maintaining inoculum under dry conditions allowed for spore viability to remain high for several years. Inoculum was viable after 4-5 years when spores were dry-stored at 4°C. To prepare inoculum for nursery inoculations, dry powder was mixed with ground peat. Inoculation of previously-disinfected seed was carried out by mixing moistened seed with the inoculum-peat mixture in a rotary mixer. A gum arabic surface adherent was added prior to mixing. Coated seeds were dried for 24 hrs. before sowing (Figure 1).

Seeds pelletized with mycorrhizal inoculum as described above were sown in glasshouse and cupola plastic container nurseries. Tree species evaluated included *Pinus pinaster* Ait., *P. pinea* L., *Quercus suber* L. and *Q. rotundifolia* Lam. Several different symbiotic fungi were evaluated as inoculum, including some which were edible.

In glasshouse experiments, growing media consisted of a mixture of sand, soil and peat. *Pinus pinea* seeds were inoculated with *Suillus bovinus* and *S. bellini* (Gnz.) Walting. Growth and vigor was greater on inoculated plants than non-inoculated ones (Figure 2)(Fonseca and Azevedo 1990, 1992). In cupola plastic nurseries, paper pot containers and sterilized commerical peat growing media were used. *Pinus pinaster* seed were inoculated with three mycorrhizal symbionts: *Pisolithus tinctorius* Minch.: Pers., *Rhizopogon luteolus* Fr.: Nordh. and *Scleroderma aurantium* Vail. Percentage of plants becoming mycorrhizal was greatest using *P. tinctorius* inoculum (80-100%), intermediate with *S. aurantium* (80%), and lowest with *R. luteolus* (50-60%). Control plants not inoculated with mycorrhizal symbionts were smaller and less vigorous than mycorrhizal plants (Azevedo 1982; Fonseca and Azevedo 1992).

Both oak species (*Q. suber* and *Q. rotundifolia*) were inoculated with two mycorrhizal symbionts: *Amanita caesarea* (Scop.:Fr.) Grev. and *Boletus aereus* Bull: Fr. Seed pelletization resulted in extensive mycorrhization; after four years, sporophores were evident near inoculated trees (*A. caesarea* on *Q. suber* (Figure 3) and *B. aereus* on *Q. rotundifolia* (Figure 4)(Fonseca and Azevedo 1994). *Quercus rotundifolia* seeds were also pelletized with Portuguese species of Tuberaceae: *Tuber lacunosum* Matt., *T. melanosporum* Vitt. and *Terfezia leonis* Tull. (Figure 5)(Azevedo 1989; Fonseca and Azevedo 1994). Two years following inoculation, mycorrhizal development was verified by examining root ultrastructure.

CONCLUSIONS

Inoculation of nursery seedlings with specific mycorrhizal symbionts is an effective way of improving outplanting performance. Effective inoculation may be obtained by pelletizing tree seed with the desired symbiont prior to sowing. Such inoculation usually results in enhanced growth and vigor of nursery stock. These low-cost techniques can be easily applied throughout the nursery industry. Unfortunately, at present such inoculation is not standard practice in Portugal nurseries. However, it is hoped that in the future more growers will adopt this system of introducing selected mycorrhizal fungi into their nursery stock.

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LITERATURE CITED

Azevedo, N.de. 1973. Micorrizas e doencas. Bol. Soc. Brot. Ser. 2, 47. p. 337-346.

Azevedo, N.de. 1982. Ectomicorrizas del *Pinus pinaster* Ait. Boletin Estacion Central Ecologia 11(22):37-42.

Azevedo, N.de. 1989. A azinheira e a tubericulura-Brana. Monografico I. Actas I Congresso de Macromixologia Galaico-Lusa. pp. 5-19.

Fonseca, N. and H. de Azevedo. 1990. Contribuction al conocimiento y control de las enfermidades del pino pinonero. Bol. San. Veg. Plagas 16:447-453.

Fonseca, N. and H. de Azevedo. 1992. Povoamentos florestais. Importancia d as ectomicorrizas na prevencao das doencas. Floresta e Ambiente 16:49-50.

Fonseca, N. and H. de Azevedo. 1994. Ectomicorrizacao e macrofungos comestiveis. IV. Congresso Galico-Luso de Macromixologia. Espanha. (unpublished).

Zak, B. 1964. Role of mycorrhizae in root diseases. Annual Review of Phytopathology. 2:377-392.

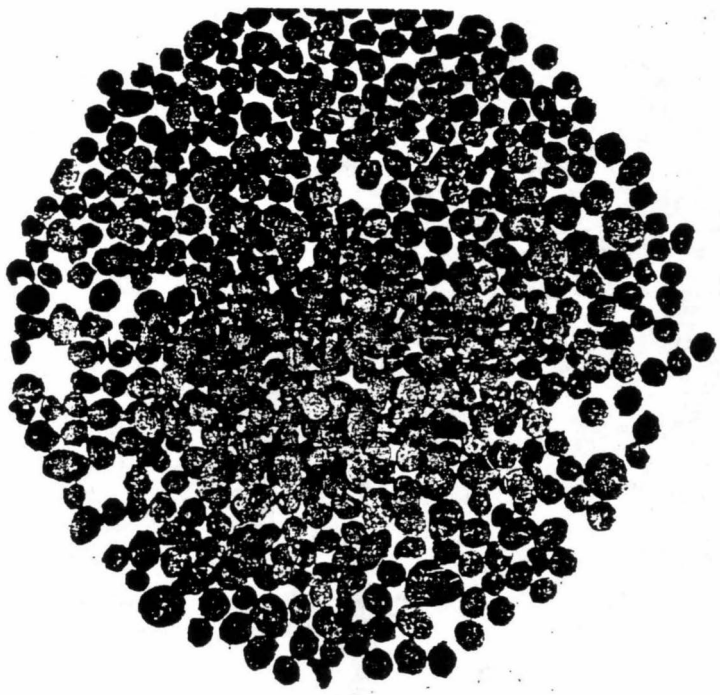

Figure 1. *Pseudotsuga* seeds pelletizied with mycorrhizal inoculum.

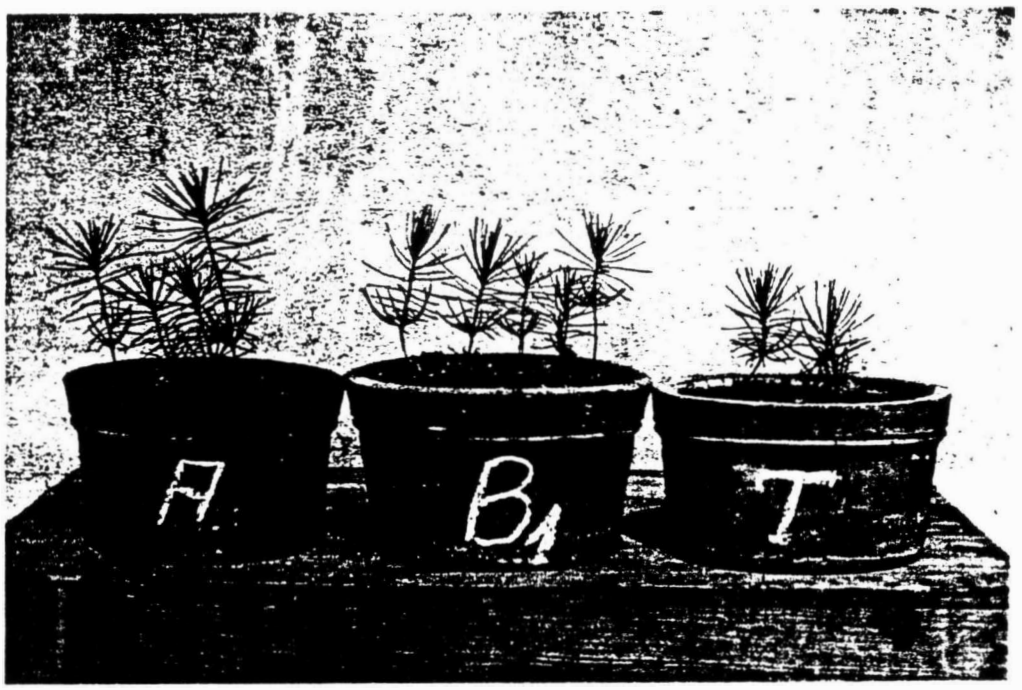

Figure 2. *Pinus pinea* seedlings inoculated with mycorrhizal symbionts: A = *Suillus bovinus*, B = *S. belinni*, T = uninoculated control.

Figure 3. Primordia carpophores of the mycorrhizal symbiont *Amanita caesarea*.

Figure 4. Primordia carpophores of the mycorrhizal symbiont *Boletus aereus*.

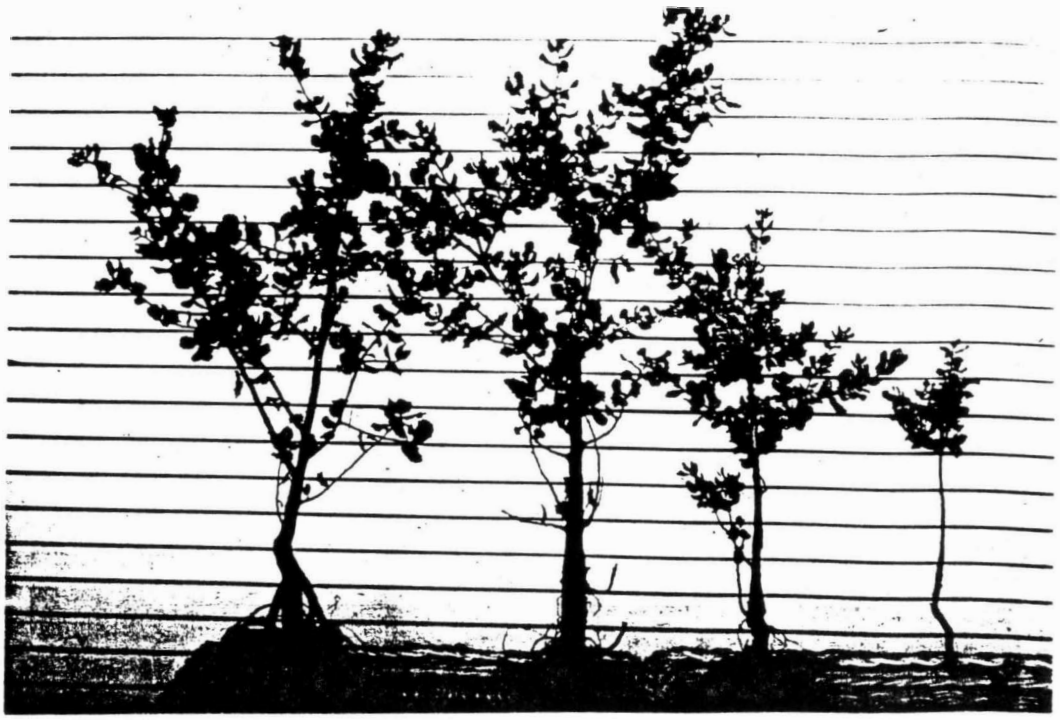

Figure 5. *Quercus rotundifolia* trees inoculated with mycorrhizal symbionts *Tuber lacunosum*, *T. melanosporum* and *Terfesia leonis* (from left to right, respectively). Uninoculated control tree on the far right.

PISOLITHUS TINCTORIUS MYCORRHIZAE INOCULATION PROVIDES LITTLE BENEFIT FOR LONGEAF AND LOBLOLLY PINES ON THE SAVANNAH RIVER SITE[1]

M.M. Cram[2], J.G. Mexal[3], and R.A. Souter[4]

ABSTRACT

Field performance of longleaf and loblolly pine inoculated with *Pisolithus tinctorius* (Pt) on 10 demonstration plantings indicated that Pt seedlings do not grow or survive better after 4-5 years than seedlings planted with naturally occurring mycorrhizae. The results also indicate that Pt-inoculation of bareroot longleaf pine can decrease survival and growth. The use of containers on two sites increased survival and growth of longleaf pine over bareroot seedlings. Height growth of container-grown longleaf pine was increased with Pt inoculation. Herbicide application did not affect longleaf pine on two sites, but did increase the growth of loblolly pine on one site.

INTRODUCTION

The presence of ectomycorrhizae on the root systems of pines is essential for seedling growth and survival (Meyer 1973; Ruehle 1982). In the southern United States, *Thelephora terrestris* (Ehrh.) ex Fr. (Tt) is the most common ectomycorrhizal fungus in nurseries following fumigation (Marx et al. 1970). When seedlings with Tt were found to perform poorly on mine reclamation sites (Marx and Artman 1979; Ruehle 1980) scientists began working with *Pisolithus tinctorius* (Pers.) Coker and Couch (Pt), an ectomycorrhizal fungus that naturally occurred on coal and kaolin spoils in the South (Marx 1977; Marx and Bryan 1975). Pine seedlings inoculated with Pt were found to survive and grow significantly better than seedlings with other mycorrhizae on mine spoils (Berry 1982; Marx and Artman 1979; Ruehle 1980; Walker et al. 1989). Encouraged by the success of Pt-inoculated seedlings for mine reclamation, Pt-inoculated seedlings were evaluated for general reforestation (Castellano and Trappe 1991; Hatchell 1985; Hatchell and Marx 1987; Kais et al. 1981; Leach and Gresham 1983; Marx 1977, 1987; Marx et al. 1988). Results from these studies have been inconsistent.

The USDA Forest Service, in cooperation with the state of South Carolina and the U.S. Department of Energy, began a 5 year project to demonstrate the best management practices to produce Pt-inoculated longleaf (*Pinus palustris* Mill.) and loblolly (*P. taeda* L.) pine for reforestation on the Savannah River Site, near Aiken, South Carolina. The 4-5-year results of this cooperative project are reported here.

METHODS

Bareroot seedlings were grown from 1987 to 1991 at the Taylor Nursery, Trenton, South Carolina. Container longleaf pine were produced in 1991 at the State Creech Seed Orchard, container facility, Wedgefield, South Carolina. All seed sources used were from either South Carolina or Georgia. The nursery beds at the Taylor Nursery were fumigated in the spring with 98% methyl bromide/2% chloropicrin. Soil fertility was adjusted with 10-10-10 fertilizer and tested for extractable phosphorus by a weak Bray analysis for optimum growing conditions (Marx 1990).

Pt was applied to seedbeds in an operational manner. Seedbeds were treated with 0.28L/m² of vegetative Pt inoculum prior to sowing (Cordell et al. 1981; Marx and Bryan 1975). The Pt inoculum was obtained from Sylvan Spawn Laboratory in 1987, and then from Mycorr Tech Inc. in 1988 to 1991 (Mycorr Tech Inc. purchased the fungus inoculum patent from Sylvan Spawn Laboratory Inc.).

[1] Paper presented at the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). Orlando-Gainesville, FL., USA. May 19-24, 1996.

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All seeds were treated with thiram prior to sowing with a precision vacuum seeder. Longleaf pine seeds were sown between March 15 and April 7 for a density of 122-133 seedlings/m^2 (Scarbrough and Allen 1954), and loblolly pine seeds were sown between April 7 and April 21 for a bed density of 267-278 seedlings/m^2.

Fungicides were applied to the seedlings to control damping-off, brown spot needle blight, and fusiform rust. Captan was applied to both species within 7 days of sowing all seedbeds (Marx and Rowan 1981). Benomyl was applied to longleaf pine 6 weeks after sowing (Kais et al. 1981), and ferbam was applied to loblolly three times per week following emergence, until July 1 of each year (Marx 1987).

Longleaf and loblolly pine seedbeds were fertilized with 4-5 applications ammonium nitrate (four applications of ammonium sulfate at equal N levels were used in 1987). Commercial potassium chloride (muriate of potash) was applied in mid-July and August of each year. In early August to October, both species were lateral-root pruned to increase the fibrosity of root systems (Hatchell 1985; Shoulders 1963).

In the fall before lifting, seedling roots were examined. Only seedbeds with a Pt index of 50 or greater (Marx et al. 1984) were used for the Pt treatment of the demonstration plantings (Cordell, personal communication). The mycorrhizal species found more often on the seedlings not inoculated with Pt (Nl) was Tt (Cordell, personal communication).

The minimum standards for a plantable longleaf pine were a root-collar diameter of 1.0cm and a root length of 15cm (White 1981); minimum standards for loblolly pine were a diameter of 0.32cm, a root length of 15cm, and a shoot height of 22.5cm. The seedlings were planted within 5 days of lifting. Container-grown longleaf pine were produced in multi-pot containers with a cavity volume of 98.32 cm^3. The medium used in containers was a mix of 40% peat, 40% vermiculite, and 20% perlite. A slow-release fertilizer (18-6-12) was added to the medium. Longleaf pine seeds were soaked for 20 min. with thiophanate-methyl, and then sown three to a cavity in April of 1991. After emergence, seedlings were thinned to one per container.

The inoculum applied to the containers were Pt spores from fruiting bodies found in the previously inoculated beds of the Taylor Nursery. Spores were applied at a rate of 0.5 g/1,000 seedlings just after seedling emergence. Thiophanate-methyl was applied every 30 days following germination. Only seedlings with a Pt index of 50 (Marx et al. 1984) or greater were used for the Pt treatment plots (Cordell, personal communication).

The 10 field planting sites were located on deep-sandy soils throughout the Savannah River Site (Table 1). Methods of site preparation varied (Table 1). All 10 sites had a mycorrhizae treatment of Pt-inoculated and Nl seedlings. The first eight sites were initially designed for a herbicide treatment of sulfometuron-methyl; however, only sites 3 and 4 received a herbicide application in March 1989. The container-grown longleaf pine were planted on sites 9 and 10 only. Treatments were randomized within eight replicated blocks for all the demonstration plantings. Seedlings were measured just after planting for an initial diameter size, and then measured again after 4 years (5 years for site 5). Differences among the means were examined for statistical significance using a randomized complete block analysis of variance (SAS 1989). An analysis of covariance was also used to determine if the variation between means was due to the initial diameter at the time of planting (sites 1-8).

RESULTS

Artificial inoculation of bareroot longleaf pine with Pt resulted in no improvement in growth (Table 2). Seedlings colonized by natural mycorrhizae performed better than Pt seedlings on 6 of 10 sites. Survival of seedlings was significantly better for Nl seedlings on sites 6-10. Spring application of sulfometuron-methyl on sites 3 and 4 had no effect on growth or survival of longleaf pine.

Use of Pt in container-grown longleaf pine increased height growth by 17-24%, but had no affect on the diameter growth after 4 years (Table 3). Container seedlings had significantly greater heights and diameters than the bareroot seedlings. Survival within the container treatment was not affected by inoculation of Pt, however, container seedlings had significantly greater overall survival than bareroot seedlings. Annosum root rot (*Heterobasidion annosum* [Fr.] Bref.) was found in dying seedlings on site 10, and was estimated to have caused an average of 6 and 2% mortality of longleaf and loblolly pine, respectively after 4 years.

Table 1. The soil series (Rogers 1990) and site preparation for sites planted with longleaf and loblolly pine seedlings.

Site	Planting Date	Soil Series	Sand Depth (cm)	Site Preparation
1	1/88	Blanton sand	120	Herbicide, chopped, burned
2	1/88	Troup sand	135	Herbicide, chopped, burned
3	1/89	Lakeland sand	200	Herbicide, chopped, burned
4	1/89	Wagram sand	55	Chopped, burned
		Blanton sand	120	Chopped, burned
5	1/90	Blanton sand	120	Sheared, raked
6	1/90	Blanton sand	120	Herbicide, chopped, burned
7	1/91	Lakeland sand	200	Herbicide, chopped, burned
8	1/91	Fuquay sand	55	Sheared, raked
		Dothan sand	18	Sheared, raked
9	1/92	Blanton sand	120	Burned, partially raked
		Lakeland sand	200	Burned, partially raked
10	1/92	Troup sand	135	Raked

Table 2. Survival and growth of longleaf pine seedlings with *Pisolithus tinctorius* and natural ectomycorrhizae after 4 years (5 years for site 5)[1].

Site	Treatment[2]	Diameter at Planting (cm)	Height (cm)	Diameter[3] (cm)	Survival percent	Seedling Volume (cm^3)	Plot Volume[4] (cm^3)
1	NI	1.02a	199a	6.0a	90a	2155a	96676a
	PT	0.92a	171b	5.8b	91a	1743b	79734b
2	NI	1.00a	150a	5.6a	81a	1381a	56084a
	PT	1.01a	164a	5.9a	92b	1649a	77936b
3	NI	1.09a	117a	4.7a	88a	881a	39655a
	PT	1.03b	110a	4.5a	88a	792a	35407a
4	NI	1.15a	142a	5.3a	84a	1303a	55426a
	PT	1.19a	147a	5.4a	89a	1394a	62361a
5	NI	1.08a	227a	3.4a	82a	2076a	84941a
	PT	1.01a	223a	3.3a	81a	2070a	83409a
6	NI	1.13a	172a	6.0a	76a	1934a	73544a
	PT	1.10a	155b	5.7b	70b	1570b	55002b
7	NI	1.02a	91a	4.6a	72a	619a	22453a
	PT	1.04a	89a	4.7a	62b	628a	19740a
8	NI	1.08a	108a	4.8a	68a	926a	32488a
	PT	1.19a	103a	4.9a	48b	909a	23006a

[1] Within planting sites, numbers followed by the same letters are not significantly different (P=0.05) as determined by analysis of variance.

[2] NI = natural ectomycorrhizae; PT = *Pisolithus tinctorius*.

[3] Diameters measured at root collar for all sites except site 5; if height exceeded 135cm, diameter was measured at breast height.

[4] Plot volume = mean seedling volume x number of surviving trees per plot.

Table 3. Survival and growth of bareroot and containerized longleaf pine seedlings with *Pisolithus tinctorius* and natural ectomycorrhizae after 4 years[1].

Site	Treatment[2]	Height (cm)	Diameter[3] (cm)	Seedling Survival Percent	Volume (cm^3)	Plot Volume[4] (cm^3)
			Bareroot			
9	NI	54a	3.7a	65a	319a	9976a
	PT	54a	3.5a	45b	297a	6824a
			Container			
	NI	60a	4.0a	90a	372a	17161a
	PT	79b	4.4a	85a	553b	23448b
			Bareroot			
10	NI	78a	4.1a	57a	503a	14340a
	PT	62b	3.9a	37b	351a	6681b
			Container			
	NI	77a	4.1a	83a	498a	20791a
	PT	93a	4.4a	80a	660a	26911a

[1] Within planting sites and container treatments, means followed by the same numbers are not significantly different (P=0.05) using analysis of variance.
[2] NI = natural ectomycorrhizae; PT = *Pisolithus tinctorius*.
[3] Diameters measured at root collar.
[4] Plot volume = mean seedling volume x number of surviving trees per plot.

Pt inoculation significantly increased initial diameter of the loblolly pine seedling on four sites (Table 4). After the 4th year, only survival on site 2 and diameter on site 5 (5th year) were greater than the NI seedlings. However, in both cases the analysis of covariance suggested that the increase could be due to the initial size differences at planting. Site 7 was the only site with increased height and diameter without the influence of initial size differences. Responses of loblolly pines to herbicide treatments on sites 3 and 4 were inconsistent. The only growth response from spring application of herbicide was found on site 3.

DISCUSSION

The presence of Pt ectomycorrhizae on root systems of longleaf and loblolly pines provided little benefit to seedling performance on deep sandy soils after 4-5 years. Although initial size of loblolly pine seedlings was often improved, growth and survival responses in the field were not consistently different than the NI seedlings. In contrast, artificial inoculation of longleaf pine seedbeds with Pt occasionally resulted in a negative growth and survival response after outplanting.

Use of Pt-inoculated seedlings for general reforestation has been found to have no consistent effect (Leach and Gresham 1983; Marx et al. 1977), and even a negative effect on seedling performance in the field (Castellano and Trappe 1991; Echols et al. 1990). Other studies have documented significant growth depressions with the use of a variety of mycorrhizae on different conifer seedlings (Bledsoe et al. 1982; Molin and Chanard 1983; Nylund and Wallander 1989; Perry et al. 1987; Stenstrom et al. 1990). Artificial inoculation with mycorrhizae can create a carbohydrate sink compared to non-mycorrhizal seedlings (Nylund and Wallander 1989) and reduce water potential (Lehto 1989). The interaction between mycorrhizae and seedlings can be negative when the mycorrhizae provides little benefit to balance its use of carbohydrates (Stenstrom et al. 1990).

Survival and growth of longleaf pines may be increased with use of containers on adverse sites (Amidon et al. 1982; Barnett 1989; Boyer 1989). Bareroot seedlings can lose 35-75% of their small roots during lifting (Rowan 1987). However, container-grown seedlings do not suffer root stripping during lifting and are able to maintain their root growth potential (South and Stumpff 1990), and avoid moisture stress better than bareroot seedlings (Becker et al. 1987).

Table 4. Survival and growth of loblolly pine seedlings with *Pisolithus tinctorius* and natural ectomycorrhizae after 4 years (5 years for site 5)[1].

Site	Treatment[2]	Diameter at Planting (cm)	Height (cm)	Diameter[3] (cm)	Survival percent	Seedling Volume (cm^3)	Plot Volume[4] (cm^3)
1	NI	0.36a	329a	3.7a	95a	2721a	129860a
	PT	0.33a	331a	3.7a	97a	2691a	131007a
2	NI	0.41a	361a	4.5a	95a	3789a	179419a
	PT	0.46b	376a	4.5a	99b	3877a	191465a
3	NI	0.33a	258a	2.7a	96a	1322a	63539a
	PT	0.36	257a	2.7a	92a	1481a	69192a
4	NI	0.36a	308a	3.8a	90a	2567a	116483a
	PT	0.40b	302a	3.7a	90a	2458a	111217a
5	NI	0.37a	382a	5.0a	90a	4992a	224988a
	PT	0.44b	395b	5.2b	90a	5401a	243104a
6	NI	0.43a	288a	3.2a	90a	2064a	94109a
	PT	0.48a	304a	3.4a	89a	2329a	104072a
7	NI	0.43a	209a	1.7a	91a	601a	27536a
	PT	0.43a	223b	2.0b	89a	758a	33299a
8	NI	0.42a	286a	3.2a	93a	2010a	94146a
	PT	0.45a	293a	3.4a	93a	2264a	105505a
9	NI	--	174a	1.3a	68a	528a	19736a
	PT	--	189a	1.5a	75a	606a	23599a
10	NI	--	235a	2.2a	87a	1027a	44963a
	PT	--	214a	1.8a	85a	745a	32804a

[1] Within planting sites, means followed by the same letter are not significantly different (P=0.05) using analysis of variance.

[2] NI = natural ectomycorrhizae; PT = *Pisolithus tinctorius*

[3] Diameters measured at root collar; when height exceeded 135cm, diameters taken at breast height.

[4] Plot volume = mean seedling volume x number of surviving trees per plot.

The addition of Pt to containerized stock resulted in increased height growth. Unfortunately, diameters of container seedlings were not measured at the time of planting, so we do not know if the average size of Pt seedlings was different than NI seedlings. Other studies have demonstrated that artificial inoculation of containers with Pt and Tt resulted in no differences in loblolly or shortleaf (*P. echinata* Mill.) pine seedling performance (Ruehle et al. 1981; Ruehle 1982). However, bareroot studies with loblolly pine showed that early presence of either Tt or Pt by artificial inoculation significantly increased size of the seedlings over natural colonization by Tt (Marx et al. 1978). These studies indicated that delay in natural colonization of containers with mycorrhizae may produce a size difference between artificially and naturally inoculated seedlings.

The herbicide application on sites 3 and 4 did not provide a benefit for longleaf pine, but did increase growth of loblolly pine on site 3. Unfortunately, only two sites received herbicide treatments, limiting our conclusions.

Use of artificial inoculation of seedlings with mycorrhizae must take into account adaptability of the fungus to a site, as well as to the tree species (Perry et al. 1987; Trappe 1977). Although Pt has been proven to increase seedling performance on mine spoils (Berry 1982; Ruehle 1980; Marx and Artman 1979; Walker et al. 1989), it has not consistently performed better than naturally-occurring mycorrhizae in nurseries (Castellano and Trappe 1991; Echols et al. 1990; Leach and Gresham 1983; Marx et al. 1977). Use of artificial inoculation is not practical if the treatment cannot produce consistent benefits to warrant the cost of application (Torrey 1992). In our study, the mycorrhizae naturally found at Taylor Nursery was adequate for seedling establishment of both longleaf and loblolly pine on the Savannah River Site.

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LITERATURE CITED

Amidon, T.E., J.P. Barnett, H.P. Gallangher and J. McGilvray. 1982. A field test of containerized seedlings under drought conditions. *In*: Guldin, R.W. and J.P. Barnett (eds.). Proceedings Southern Containerized Forest Tree Seedling Conference. USDA Forest Service, General Technical Report SO-37. pp. 139-144.

Barnett, J.P. 1989. Site preparation, containers, and soil types affect field performance of loblolly and longleaf pine seedlings. *In*: Miller, J.H. (ed.). Proceedings 5th Biennial Southern Silviculture Research Conference. USDA Forest Service, General Technical Report SO-74. pp. 155-158.

Bledsoe, C.S., K. Tennyson and W. Lopushinsky. 1982. Survival and growth of outplanted Douglas-fir seedlings inoculated with mycorrhizal fungi. Canadian Journal of Forest Research 12:720-723.

Becker, C.A., G.D. Mroz and L.G. Fuller. 1987. The effects of plant moisture stress on red pine (*Pinus resinosa*) seedling growth and establishment. Canadian Journal of Forest Research 17:813-820.

Berry, C.R. 1982. Survival and growth of pine hybrid seedlings with *Pisolithus* ectomycorrhizae on coal spoils is Alabama and Tennessee. Journal of Environmental Quality 11:709-715.

Boyer, W.D. 1989. Response of planted longleaf pine bare-root and container stock to site preparation and release: fifth-year results. *In*: Miller, J.H. (ed.). Proceedings 5th Biennial Southern Silviculture Research Conference. USDA Forest Service, General Technical Report SO-74. pp. 165-168.

Castellano, M.A. and J.M. Trappe. 1991. *Pisolithus tinctorius* fails to improve plantation performance of inoculated conifers in southwestern Oregon. New Forests 5:349-358.

Cordell, C.E., D.H. Marx, J.R. Lott and D.S. Kenney.1981. The practical application of *Pisolithus tinctorius* ectomycorrhizal inoculum in forest tree nurseries. *In*: Forest Regeneration, American Society of Agricultural Engineerings, Symposium on Engineering Systems for Forest Regeneration, Raleigh, NC. pp. 38-42.

Echols, R.J., C.E. Meier, A.W. Ezell and C.R. McKinley. 1990. Dry site survival of bareroot and container seedlings of southern pines from different genetic sources given root dip and ectomycorrhizal treatments. Tree Planters' Notes 41(2):13-21.

Hatchell, G.E. 1985. Seedling quality and field performance of longleaf pine seedlings affected by ectomycorrhizae and nursery cultural practices. *In*: Shoulders, E. (ed.). Proceedings 3rd Biennial Southern Silviculture Research Conference. USDA Forest Service, General Technical Report SO-54. pp. 395-402.

Hatchell, G.E. and D.H. Marx. 1987. Response of longleaf, sand, and loblolly pines to *Pisolithus* ectomycorrhizae and fertilizer on a sandhills site in South Carolina. Forest Science 33:301-315.

Kais, A.G., G.A. Snow and D.H. Marx. 1981. The effects of benomyl and *Pisolithus tinctorius* ectomycorrhizae on survival and growth of longleaf pine seedlings. Southern Journal of Applied Forestry 5:189-194.

Leach, G.N. and H.H. Gresham. 1983. Early field performance of loblolly pine seedlings with *Pisolithus tinctorius* ectomycorrhizae on two lower coastal plain sites. Southern Journal of Applied Forestry 7:149-153.

Lehto, T.H. 1989. Effects of mycorrhiza and drought on photosynthesis and water relations of Sitka Spruce. Agricultural Ecosystems and Environment 28:299-303.

Marx, D.H. 1977. Tree host range and world distribution of the ectomycorrhizal fungus *Pisolithus tinctorius*. Canadian Journal of Microbiology 23:217-223.

Marx, D.H. 1987. Triadimefon and *Pisolithus* ectomycorrhizae affect second-year field performance of loblolly Pine. USDA Forest Service Research Note SE-349. 6p.

Marx, D.H. 1990. Soil pH and nitrogen influence *Pisolithus* ectomycorrhizal development and growth of loblolly pine seedlings. Forest Science 36:224-245.

Marx, D.H. and J.D. Artman. 1979. *Pisolithus tinctorius* ectomycorrhizae improve survival and growth of pine seedlings on acid coal spoils in Kentucky and Virginia. Reclamation Reviews 2:23-31.

Marx, D.H. and W.C. Bryan. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. Forest Science 21:245-254.

Marx, D.H., W.C. Bryan and C.E. Cordell. 1977. Survival and growth of pine seedlings with *Pisolithus* ectomycorrhizae after two years on reforestation sites in North Carolina and Florida. Forest Science 23:363-373.

Marx, D.H., W.C. Bryan and L.F. Grand. 1970. Colonization, isolation, and cultural descriptions of *Thelephora terrestris* and other ectomycorrhizal fungi of shortleaf pine seedlings grown in fumigated soil. Canadian Journal of Botany 48:207-211.

Marx, D.H., C.E. Cordell and A. Clark, III. 1988. Eight-year performance of loblolly pine with *Pisolithus* ectomycorrhizae on a good-quality forest site. Southern Journal of Applied Forestry 12:275-280.

Marx, D.H., C.E. Cordell, D.S. Kenney, J.G. Mexal, J.D. Artman, J.W. Riffle and R.J. Molina. 1984. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on bare-root tree seedlings. Forest Science Monograph 25. 101p.

Marx, D.H., W.G. Morris and J.G. Mexal. 1978. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated and nonfumigated nursery soil infested with different fungal symbionts. Forest Science 24:193-203.

Marx, D.H. and S.J. Rowan. 1981. Fungicides influence growth and development of specific ectomycorrhizae on loblolly pine seedlings. Forest Science 27:167-176.

Meyer, F.H. 1973. Distribution of ectomycorrhizae in native and man-made forests. *In*: Marks, G.C. and T. Kozlowski (eds.). Ectomycorrhizae: Their Ecology and Physiology. Academic Press, New York. pp. 79-101.

Molin, A.R. and J. Chanard. 1983. Use of the ectomycorrhizal fungus *Laccaria laccata* in Forestry. II. Effects of fertilizer forms and levels on ectomycorrhizal development and growth of container-grown Douglas-fir and ponderosa pine seedlings. Canadian Journal of Forest Research 13:89-95.

Nylund, J.E. and H. Wallander. 1989. Effects of ectomycorrhiza on host growth and carbon balance in a semi-hydroponic cultivation system. New Phytologist 112:389-398.

Perry, D.A., R. Molina and M.P. Amaranthus. 1987. Mycorrhizae mycorrhizospheres and reforestation: current knowledge and research needs. Canadian Journal of Forest Research 17:929-940.

Rogers, V.A. 1990. Soil Survey of Savannah River Plant Area, Parts of Aiken, Barnwell, and Allendale Counties, South Carolina. U.S. Department of Agriculture, Soil Conservation Service. 127p.

Rowan, S.J. 1987. Nursery seedling quality affects growth and survival in outplantings. Georgia Forest Research Paper 70. Gorgia Forestry Commission, Macon. 15p.

Ruehle, J.L. 1980. Growth of containerized loblolly pine with specific ectomycorrhizae after 2 years on an amended borrow pit. Reclamation Reviews 3:95-101.

Ruehle, J.L. 1982. Field performance of container-grown loblolly pine seedlings with specific ectomycorrhizae on a reforestation site in South Carolina. Southern Journal of Applied Forestry 6:30-33.

Ruehle, J.L., D.H. Marx, J.P. Barnett and W.H. Pawuk. 1981. Survival and growth of container-grown and bare-root shortleaf pine seedlings with *Pisolithus* and *Thelephora* ectomycorrhizae. Southern Journal of Applied Forestry 5:20-24.

SAS Institute, Inc. 1989. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2, Cary, NC: SAS Institute Inc., 846p.

Scarbrough, N.M. and R.M. Allen. 1954. Better longleaf seedlings from low-density nursery beds. Tree Planters' Notes 18:29-32.

Shoulders, E. 1963. Root-pruning southern pines in the nursery. USDA Forest Service, Research Paper. SO-5, 6p.

South, D.B. and N.J. Stumpff. 1990. Root stripping reduces root growth potential of loblolly pine seedlings. Southern Journal of Applied Forestry 14:196-199.

Stenstrom, E., M. Ek and T. Unestam. 1990. Variation in field response of *Pinus sylvestris* to nursery inoculation with four different ectomycorrhizal fungi. Canadian Journal of Forest Research 20:1796-1803.

Torrey, J.G. 1992. Can plant productivity be increased by inoculation of tree roots with soil microorganisms? Canadian Journal of Forest Research 22:1815-1823.

Trappe, J.M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annual Review of Phytopathology 15:203-222.

Walker, R.F., D.C. West, S.B. McLaughlin and C.C. Amundsen. 1989. Growth, xylem pressure potential, and nutrient absorption of loblolly pine on a reclaimed surface mine as affected by an induced *Pisolithus tinctorius* infection. Forest Science 35:569-581.

White, J.B. 1981. The influence of seedling size and length of storage on longleaf pine survival. Tree Planters' Notes 32:3-4.

EVALUATION OF FUNGI FOR BIOCONTROL OF CYLINDROCLADIUM SCOPARIUM, RHIZOCTONIA SOLANI AND BOTRYTIS CINEREA TO BE USED IN SUSPENDED BRAZILIAN EUCALYPTUS NURSERIES[1]

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ABSTRACT

Six selected *Trichoderma* isolates belonging to five species were tested for 15 and 30 days' incubation in a substrate of Eucalyptus bark compost plus soil (8:2 w/w) as a control of seedling damping-off of *Eucalyptus grandis* incited by *Cylindrocladium scoparium* and *Rhizoctonia solani*. All isolates effectively controlled both pathogens as determined by seedling survival rate for 15 or 30 days after seedling. Survival rates were equal to or greater than those resulting from fungicide treatment. Mechanisms involved in *C. scoparium* control were antibiosis and competition for colonization niches and nutrients, whereas competition and parasitism were involved in suppression of *R. solani*. *Gliocladium roseum, Penicillium* sp., *Trichoderma harzianum, T. viride* and two unidentified isolates of *Trichoderma* were tested under nursery and growth-room conditions as biocontrol agents for *Botrytis cinerea* on 70- to 80-day old *E. grandis* plants. Suppressiveness efficacy varied widely. In a growth-room assay, pre-inoculation seedling stress (30°C/60 hr./dark) and pathogen inoculation 24 hr. before antagonists or fungicide applications were excessively favorable to pathogen attack. As a consequence, there were no significant differences among any treatments which had more than 80% of seedlings attacked by *B. cinerea*. In a second growth-room assay, *G. roseum* treatment significantly suppressed the pathogen. Other treatments, including fungicides, did not differ from one another nor were they effective in controlling disease.

INTRODUCTION

In Brazilian Eucalyptus nurseries managed by direct seeding into suspended containers, soil-borne pathogens have been controlled by hygenic treatments to remove pathogens from seeds, trays, containers, growing media, and water (Ferreira 1991). Such a production system has resulted in healthy seedlings without fungicide use. *Cylindrocladium scoparium* Morg. and *Rhizoctonia solani* Kuehn may accidentally be introduced during seedling production, particularly by water contamination. In that case, biological control may be useful.

Botrytis cinerea Pers. ex Fr. is frequently introduced into nurseries as air-borne inoculum (Ferreira 1989; Souza 1991). Biocontrol of this pathogen has been recommended to replace manual cultural measures which have been efficient but unfeasible in larger nurseries (Ferreira 1991, 1994). Biocontrol may also substitute for fungicide applications which may result in inconsistent efficacy and induction of pathogen resistance to chemicals (Ghini and Krugner 1987; Sutherland et al. 1989; Sutton et al. 1995).

In the present paper, we report results of research on biological control of these three pathogens.

MATERIALS AND METHODS

Biocontrol of *Cylindrocladium scoparium* and *Rhizoctonia solani*:

Nineteen *Trichoderma* spp. isolates were obtained from organic compost and peat from either tropical (T) or sub-tropical (S) Brazilian locations using mycelial baits of *R. solani* (Garcia 1995). *In vitro* tests were undertaken to evaluate competition, antibiosis and parasitism of each *Trichoderma* isolate against *C. scoparium* and *R. solani*. Six of the most promising isolates were selected for inclusion in disease suppressiveness tests. Growing media consisting of sterilized organic compost of *Eucalyptus* and soil (8:2 w/w) was infested with each of the selected *Trichoderma* isolates (106 spores/ml; 50ml of spore suspension per liter of media). After incubation at 25°C (T isolates) or 20°C (S isolates) for 15 or 30 days, media were infested with *C. scoparium* (100

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conidia/cm²) or crushed mycelium of *R. solani* (10 ml/cm²; 1g of mycelia per liter of media). Fungal-media mixtures were incubated another 4 days at 25°C. Each treatment replicate consisted of 500 cm2 of fungal-media mixture. Each replicate was seeded with 150 *Eucalyptus grandis* Hill ex. Maid. seeds (150 seeds/500m2). The experiment was conducted in growth rooms at 25°C and 12-hr. photoperiods. Twelve days after seeding, number of emerged seedlings per replication/treatment were counted. The experiment was then moved to a greenhouse where the same evaluation was conducted 16 days later.

Biocontrol of *Botrytis cinerea*:

One isolate each of *Penicillium* sp., *Trichoderma harzianum* Rifai, *T. viride* Pers. ex Fr. and two unidentified *Trichoderma* spp. as well as an isolate of *Gliocladium roseum* (Lk. ex Fr.) Bain., an effective pathogen suppressant on other hosts obtained from strawberry (Sutton et al. 1995), were tested as biocontrols of *B. cinerea* on *E. grandis* seedlings. Three sets of experiments were conducted: (a) two pathogen inoculations (104 conidia/ml) separated by a 2-week interval were followed after 24 hr. by either antagonists (107 conidia/ml) or fungicide applications (35g benomyl plus 100g captan/100 liter of water); these tests were conducted in the nursery. (b) seedlings were initially exposed to stress (30°C for 60 hr. in the dark) in growth rooms; after stress, seedlings were inoculated with the pathogen (106 conidia/ml), incubated in a moist chamber for 24 hr. (21°C - 12 hr. photoperiod), followed by either inoculation with antagonists (107 conidia/ml) or exposure to the fungicide mixture, and reincubated for another 4 days. (c) the same treatment as (b), but without pre-inoculation stress and applications of either antagonists or fungicides 48 hr. before pathogen inoculation and increasing incubation periods in moist chambers from 4 to 6 days.

RESULTS AND DISCUSSION

Biocontrol of *Cylindrocladium scoparium* and *Rhizoctonia solani*:

All *Trichoderma* isolates were effective against both pathogens regardless of incubation periods or evaluation times (Tables 1 and 2). Results are presented for only the 15-day incubation period and the first evaluation. For both pathogens, biocontrol treat-

Table 1. Effects of *Trichoderma* spp. and fungicide treatments on control *Cylindrocladium scoparium* on *Eucalyptus grandis* seedling

Treatment	Percent Seedling Survival[1]	
T. crassum (isolate a) + *C. scoparium*	106a[2]	---
T. crassum (isolate b) + *C. scoparium*	90a	---
Unidentified *Trichoderma* + *C. scoparium*	99a	---
T. harzianum + *C. scoparium*	---	94a[2]
T. koningii + *C. scoparium*	---	83a
T. viride + *C. scoparium*	---	93a
C. scoparium alone	58b	14b
NSEBC + SSo[3] + *C. scoparium*	101a	83a
SS + NR[4] + *C. scoparium*	24c	88a
SS + fungicide[5] + *C. scoparium*	94a	94a
Control[6]	100	100
CV%[7]	12.4	10.0

[1]Percentage of *E. grandis* seedlings surviving 12 days after seeding into a growing medium composed of organic compost of Eucalyptus bark and soil.
[2] Within each column, means followed by the same letter are not significantly different (P=0.01) using Tukey's test.
[3] Growing media composed of non-sterilized Eucalyptus bark compost and sterilized soil.
[4] Sterilized growing media exposed to natural infestation outside for 15 days.
[5] Sterilized growing media and application of fungicide (35g benomyl + 100g captan/100 liters water immediately after seeding and 15 days later).
[6] Sterilized growing media.
[7] Coefficient of variation.

ments provided disease control that was at least equal to fungicide treatments. One of the treatments involved subjecting sterilized media to natural inoculum outside (SS + NR). This treatment was also effective for *C. scoparium* suppression, but only when reinfestation occurred predominantly with *Trichoderma* sp. It was noted that when natural reinfestation of growing media by *Monilia* sp. occurred, disease suppression failed. In tests of both pathogens, the Eucalyptus bark compost used as growing media provided some level of natural suppressiveness to each pathogen. Also, if the growing media were not sterilized prior to seeding with Eucalyptus, very poor seedling growth and higher seedling mortality often occurred.

Additional laboratory tests (data not given) indicated that the major mechanisms of biocontrol against *C. scoparium* involved antibiosis and competition for colonization niches and nutrients. Similar tests against *R. solani* indicated that competition and mycoparasitism were major mechanisms of biocontrol.

Biocontrol of *Botrytis cinerea*:

Extensive experimental variability occurred in biocontrol tests of *B. cinerea* in the nursery. In growth room experiments, stressing seedlings resulted in extremely favorable conditions for infection by the pathogen. As a result, no significant differences were attributed to biocontrol agents when compared with nontreated controls (Table 3). However, in the test involving *G. roseum*, this fungus provided significantly greater control (as manifested by percent seedlings infected) than other biocontrol agents and fungicide treatments. We noticed that *G. roseum* readily colonized tissues injured by stress treatments.

Table 2. Effects of *Trichoderma* spp. and fungicide treatments on control of *Rhizoctonia solani* on *Eucalyptus grandis* seedlings.

Treatment	Percent Seedling Survival[1]
T. crassum (isolate a) + *R. solani*	112a[2]
T. crassum (isolate b) + *R. solani*	105a
Unidentified *Trichoderma* + *R. solani*	73a
T. harzianum + *R. solani*	105a
T. koningii + *R. solani*	101a
T. viride + *R. solani*	104a
R. solani alone	28b
NSEBC + SSo[3] + *R. solani*	102a
SS + NR[4] + *R. solani*	29b
SS + fungicide[5] + *R. solani*	41c
Control[6]	100
CV%[7]	14.4

[1]Percentage of E. grandis seedlings surviving after seedling into a growing medium composed of organic compost of Eucalyptus bark and soil.

[2] Within the column, means followed by the same letter are not significantly different (P=0.01) using Tukey's test.

[3] Growing media composed of non-sterilized Eucalyptus bark compost and sterilized soil.

[4] Sterilized growing media exposed to natural infestation outside for 15 days.

[5] Sterilized growing media and apllication of fungicide (35g benomyl + 100g captan/100 liters water immediately after seeding and 15 days later).

[6] Sterilized growing media.

[7] Coefficient of variation.

Table 3. Effects of selected *Trichoderma, Penicillium* and *Gliocladium* isolates on control of *Botrytis cinerea* on *Eucalyptus grandis* seedlings.

Treatment	Percent Seedlings Infected[1]
Unidentified *Trichoderma* (a)	97a[2]
Unidentified *Trichoderma* (b)	80a
Trichoderma viride	94a
Trichoderma harzianum	92a
Gliocladium roseum	60b
Unidentified *Penicillium*	93a
Fungicide[3]	85a
Control[4]	85a
CV%[5]	7.2

[1]Percentage of *E. grandis* seedlings attacked by *B. cinerea* in a growth room 78 days after seeding; antagonists and fungicides were applied 70 days after seeding and *B. cinerea* was inoculated 48 hr. later.
[2] Within the column, means followed by the same letter are not significantly different (P=0.01) using Tukey's test.
[3] Fungicide consisted of 35g benomyl + 100g captan/100 liters of water.
[4] No biocontrol or fungicide applied.
[5] Coefficient of variation.

Conclusions:

Our tests showed that several isolates of *Trichoderma* and *Gliocladium roseum* were effective biocontrol agents against important Eucalyptus pathogens such as *Cylindrocladium scoparium, Rhizoctonia solani*, and *Botrytis cinerea*. Most of these biocontrol agents were at least as effective as standard fungicides commonly used in nurseries. Therefore, we feel that biological control can effectively contribute to growing healthy Eucalyptus seedlings in Brazilian nurseries without fungicides.

LITERATURE CITED

Ferreira, F.A. 1989. Patologia Florestal. Principais doencas florestais no Brasil. Vicosa, UVV, SIF. 570p.

Ferreira, F.A. 1991. Controle integrado do tombamento de mudas de eucalipto e do apodrecimento de estacas para enraizamento. Fitopatologia Brasileira 16(2):VII.

Ferreira, F.A. 1994. Control of eucalyptus nursery seedlings in Brazil: 1990-1993. In: Perrin, R. and J. R. Sutherland (eds.). Diseases and Insects in Forest Nuseries. Dijon (France), October 3-10, 1993. Institut National De La Recherche Agronomique. Les Colloques No. 68. pp 315-320.

Garcia, M.C.M. 1995. Selecao de *Trichoderma* spp. visando ao controle biologico de *Cylindrocladium scoparium* e *Rhizoctonia solani* em viveiros suspensos de eucalipto. Viscosa, UFV. (M.S. Thesis).

Ghini, R. and T.L. Krugner. 1987. Ocorrencia de Botrytis cinerea resistente a benomil em viveiro de Eucalyptus viminalis, em Tres Barras, Sc. Summa Phytopathologica 13(1-1):36.

Souza, M.G. 1991. Etiologia e controle do tombamento de mudas de eucalipto, causado por Botrytis cinerea, no estadio de fechamento de canteiros. Vicosa, MG, UFV, Imprensa Universitaria. 53p.

Sutherland, J.R., G.M. Shrimpton and R.N. Sturrock 1989. Diseases and insects in British Columbia forest seedling nurseries. Canadian Forestry Service, British Columbia Ministry of Forests: Economic & Regional Agreement, FRA Report 065. 85p.

Sutton, J.C., P.G. Zhang and A.A. Hopkin. 1995. Alternative biological and biorational control of Botrytis gray mold in containerized conifer stock. Canadian Forestry Service, NODA/ Northern Forestry Program. Final Report. 96p.

THE APPLICATION OF MICROBIOLOGICAL ANATAGONISM FOR BIOLOGICAL CONTROL OF DAMPING-OFF IN NURSERIES[1]

T.I. Gromovykh[2], A.L. Malinovsky[2], B.M. Gukasyan[2],
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ABSTRACT

Many different types of plant pathogenic fungi are responsible for losses of pine and larch seedlings in Siberian forest nurseries. Several species in the genus *Fusarium* are the most important pathogens. Maximum disease losses occur on first and second-year seedlings in June and August. Many isolates of fungal and bacterial antagonists were screened for their potential to control important nursery pathogens. One isolate of *Trichoderma harzianum* (isolate U) and one of *Pseudomonas fluorescens* (isolate 94) displayed the greatest biocontrol potential. Formulations of these organisms were prepared and designated Trichodermin-C and Rizo-94, respectively. Application of these biocontrol agents onto conifer seed resulted in increased germination and reduced seedling losses. Trichodermin-C is preferable to mass applications of antagonists because it maintains its biocontrol activity for a long time and is easy to produce, transport, and apply. We have developed techniques for mass production of these biocontrol formulations that can be applied widely in nurseries to reduce disease loses.

INTRODUCTION

Coniferous plants in Russian Siberia have great importance both in forestry and ecology of the region. There is great need for coniferous seedlings for reforestation of large tracts of forest land. An integrated system of conifer seedling production in forest nurseries is currently being developed which emphasizes minimum application of chemical pesticides (Rodzestvensky and Kleshnin 1980). Therefore, biological control using antagonistic microorganisms is receiving special attention (Gromovykh 1983a; Zavelishko and Nikolaeva 1990).

The most important pathogenic organisms in Siberian forest nurseries are fungi which reside in soil and plant residues for extended periods (Yakimenko 1992) (Table 1). Coniferous seed in Siberia may also be infected by phytopathogenic organisms (Table 1). For example, pathogenic organisms may reside in *Pinus sylvestris* L. and *Larix silbirica* (Ledeb) seedlots at levels of about 25% and 69%, respectively.

First- and second-year pine and larch seedlings are especially damaged by fungi (Bily 1988). The most important phytopathogenic fungi are in the genus *Fusarium*, which comprises more than 65% of all nursery pathogens (Gromovyky 1983b). Species of *Alternaria* and *Botrytis* are next in importance; *Verticillium* and *Pythium* species are less commonly encountered and seem restricted to nurseries in southern regions. Most of these fungi are important seed pathogens, often causing severe damping-off losses. Damping-off occurs most commonly in the second half of June and usually decreases in July (Gromovyky 1983a). A new wave of disease can occur the second half of August, particularly if seedling stems have remained succulent and not lignified.

For purposes of developing effective biocontrol agents to combat damping-off in nurseries, we investigated a large number of fungal isolates in the genus *Trichoderma* (Ghisalberti and Sivasithamparam 1991) and potential bacterial antagonists in the genera *Bacillus* and *Pseudomonas*.

METHODS

Initially, several *Trichoderma*, *Pseudomonas*, and spore-forming *Bacillus* isolates were screened for antagonistic activity against the major damping-off pathogens of pine and larch seedlings. We used the paper disk method of quantifying antagonistic activity (Yodzef 1983). Briefly, this procedure involved growing *T. harzianum* in liquid Czpek medium for 15 days, after which the fungal mycelium was removed by passing the liquid through a membrane filter. This liquid extract was used to moisten sterile 5mm

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Table 1. Important phytopathogenic fungi occurring in Siberian nursery soils and on pine and larch seed.

Fungal Species	Range of Soil Population (cfu/g)[1]	Percent Infection of Selected Seedlots[2]	
		Pinus spp.	*Larix* spp.
Alternaria alternata	300-500	19.3±3.4	35.0±4.5
Alternaria tenuisima	100-300	8.3±1.2	11.7±1.4
Botrytis cinerea	500-700	3.8±0.7	9.2±0.7
Fusarium avenaceum var. *herbarum*	300-500	4.5±0.8	17.4±2.3
Fusarium graminearum	300-500	6.7±0.9	13.5±2.6
Fusarium heterosporum	100-300	9.3±1.2	16.8±3.0
Fusarium moniliforme	300-500	13.2±2.3	18.5±3.4
Fusarium oxysporum var. *orthoceras*	500-700	10.8±1.6	11.5±2.2
Fusarium solani var. *argillaceum*	500-700	16.4±3.5	14.3±2.8
Fusarium sporotrichioides var. *sporotrichioides*	700-1000	22.7±3.5	30.6±3.2
Pythium debaryanum	0-100	10.5±1.6	22.0±2.4
Verticillium albo-atrum	0-100	7.5±1.2	8.3±0.9

[1] Colony-forming units per gram of soil.
[2] Means followed by standard error.

paper disks. After moistening, the disks were dried and placed onto agar cultures of test pathogenic fungi. These plates were intially placed under refrigeration (10°C) for 10 hrs. and then incubated at 28°C for 24 hrs. After incubation, width of the inhibition zones were measured. Strong inhibition was defined as greater than 30mm; average inhibition from 15-20mm, and weak inhibition below 15mm. This experiment was replicated 5 times for each test antagonist with each pathogenic fungus.

After microorganism screening, preparations of the best biocontrol candidates for experimentation were prepared. The fungal biocontrol preparation was called "Trichodermin-C". It consisted of a pure spore preparation of *T. harzianum* (isolate U), with spores making up 1% of the mixture. This preparation was easy to prepare and maintained viability for long time periods (Tulpanova et al. 1995). It was also easily applied to seed and seedlings. The bacterial biocontrol preparation was called "Rizo-94." It consisted of a preparation of *P. fluorescens* (isolate 94) cells suspended in succinate sodium medium with 1% casein added. Bacterial cell concentration was about $1X10^9$ cells/ml. This material remained viable in suspension for at least 7 days.

Trichodermin C and Rizo 94 were evaluated for effects on root colonization by pathogenic fungi. Pine and larch seedlings were grown in a phytotron. Before sowing, seed were treated with the two biocontrol preparations. Treatments were evaluated for effects on quantity of pathogenic fungi on roots (State Standard 12.044-81 1981). Trichodermin-C was applied to seed as a powder at the rate of 2g/kg of seed. Other seeds were treated with Rizo-94 by spraying 100ml of the suspension onto 1kg of seed. Seed treated with biocontrol agents and untreated controls were sown onto ceramzite treated with 0.2% KOH and KH2PO4 at 0.75g/L. Plants were grown in a phytotron kept at 25°C and relative humidity of 74-75%. Natural infection by pathogenic fungi occurred.

Field tests were also conducted to evaluate efficacy of Trichodermin-C and Rizo-94 biocontrol preparations. Tests were conducted during 1994 and 1995 in several Siberian forest nurseries under conditions of natural infection by pathogens. *P. sylvestris* and *L. sibirica* seed were treated with biocontrol preparations and sown using standard nursery practice. As a control, some seeds were treated with a 5% solution of KMnO4, which is the standard practice for forest tree seed in Russia (State Standard of the USSR 1988). Seedling emergence was

monitored for 120 days following sowing and seedling damping-off determined.

RESULTS AND DISCUSSION

An isolate of *T. harzianum* Rifai designated "U" was found to be most anagonistic toward pathogenic fungi (Figure 1). Likewise and isolate of *P. fluorescens* designated "94" showed the most promise as an bacterial antagonist toward the pathogens (Figure 1).

Pre-treatment of pine and larch seed with biocontrol preparations of *T. harzianum* and *P. fluorescens* resulted in reduced colonization of roots by potential pathogenic fungi in the genera *Fusarium* and *Alternaria* (Figure 2). For example, percent of *Fusarium* spp. isolated from pine roots was reduced by 60% when Trichodermin C was applied to seed prior to sowing. Likewise, these potential pathogens were reduced by 78% on larch roots when seed was similarly treated. Changes in levels of *Botrytis cinerea* and *Pythium* spp. were also evident in Figure 2 and included in "others". We also found that populations of *Cladosporium* and *Aspergillus* were reduced by application of the biocontrol agents, whereas levels of *Penicillium* and *Trichoderma* increased on roots of seedlings produced from biocontrol-treated seed. We were able to determine that *T. harzianum* (isolate U) remained viable on inoculated plant roots for 45-50 days and therefore provided protection against pathogenic fungi for prolonged periods. Reisolation of *T. harzianum* only occurred in treatments in which Trichomerdin-C were applied; therefore we were sure that the reisolated *Trichoderma* was the biocontrol organism.

Rizo-94 was also very effective in reducing root colonization by potentially- pathogenic fungi (Figure 2). Application of this biocontrol preparation resulted in increased germinative energy of pine (44.5%) and larch (42.2%) seed and acceleration of seedling growth. Germinative energy was defined as the number of seeds that normally germinated within seven days (State Standard of the USSR 1988).

Effects of the two biocontrol preparations on performance in nurseries are summarized in Figure 3. Basically, seed treatment with both biocontrol preparations decreased seedling mortality due to post-emergence damping-off.

CONCLUSIONS

Preparations of *T. harzianum* and *P. fluorescens* were effective biocontrol agents when applied to pine and larch seed prior to sowing in Siberian forest nurseries. Biocontrol agents showed antagonism toward a wide range of potentially pathogenic fungi, reduced root colonization by important pathogens and improved seedling survival in nursery soil. Large quantities of these biocontrol agents can be prepared and easily applied to improve seed germination, seedling performance, and reduce damping- off losses in nurseries (Tulpanova et al. 1995).

LITERATURE CITED

Bily, V.I. (ed.). 1988. [Microorganims-pathogens of plants]. Science Duma, Kiev. pp. 141-147 (in Russian).

Ghisalberti, E.L. and K. Sivasithamparam. 1991. Antifungal antibiotics produced by *Trichoderma* spp. Soil Biology and Biochemistry 23:1011-1020.

Gromovykh, T.I. 1983a. [Microbe antagonists and biological sterilization of soils in forest nurseries. Microorganisms in forest biocenosis of Siberia]. Publishing House of the Institute of Forests, Krasnoyarsk. pp. 81-84 (in Russian).

Gromovyky, T.I. 1983b. [The effect of microbe-parasites of *Fusarium* genus on the seed germination of *Pinus sylvestris*. Microbe associations in forest biocenosis]. Publishing House of the Institute of Forests, Krasnoyarsk. pp. 56-59 (in Russian).

Rodzestvensky, V.I. and A.D. Kleshnin. 1980. [Controlled cultivation of plants in the artificial environment]. Publishing House Science. 200p. (in Russian).

State Standard of the USSR. 1988. [The seeds of trees and bushes. The rules of sample selection and the methods of determining of sowing quality of seeds]. Science: State Standard of the USSR. pp. 3-27. (in Russian).

State Standard 12.044-81. 1981. [Methods for determining the level of disease infection]. Science: Publishing House of Standards. pp. 5-7. (in Russian).

Tulpanova, V.A., T.I. Gromovykh, A.L. Malinovsky, T.I. Kozlova, I.A. Arefyeva and A.V. Nechaev. 1995. The biotechnology of mass production of spores and its application as a biological seed treatment. International Conference of Fundamental and Applied Problems of Environmental Protection. Sept. 12-16, 1995. Tomsk. 2:103 (Abstract).

Yakimenko, E.E. 1992. [Soil fungi in forest nurseries]. Journal of Mycology and Phytopathology 26:480-486. (in Russian).

Yodzef, S. 1983. [Methods of soil microbiology]. Publishing House Ear, Moscow. pp. 187-193. (in Russian).

Zavelishko, I.A. and S.I. Nikolaeva. 1990. [Evaluation of antibiotic activity of mycelium fungi in respect to fungus phytopathogens]. Journal of Microbiology 52:89-91. (in Russian).

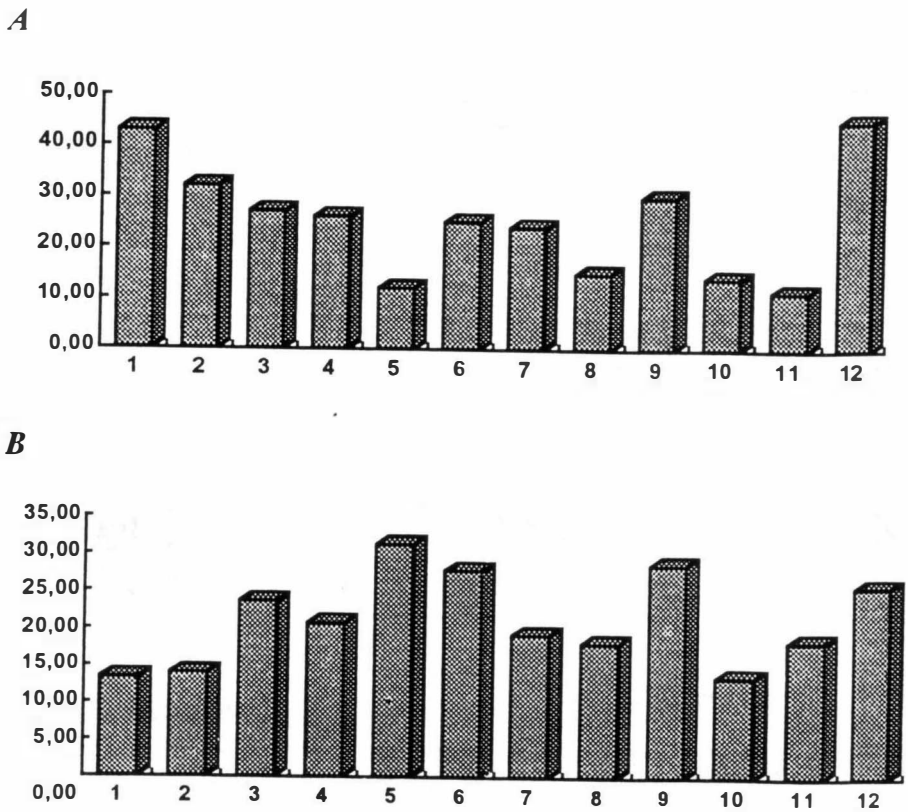

Figure 1. Antagonistic activity of *Trichoderma harzianum* (isolate U) and *Pseudomonas fluorescens* (isolate 94) on selected potentially pathogenic fungi. Pathogens evaluated: 1. *Alternaria alternata*, 2. *Alternaria tenuisima*, 3. *Fusarium avenaceum* var. *herbarium*, 4. *Fusarium graminearum*, 5. *Fusarium heterosporum*, 6. *Fusarium moniliforme*, 7. *Fusarium oxysporum* var. *orthoceras*, 8. *Fusarium solani* var. *agrillaceum*, 9. *Fusarium sporotrichioides* var. *sporotrichioides*, 10. *Pythium debaryanum*, 11. *Verticillium albo-atrum*, 12. *Botrytis cinerea*.

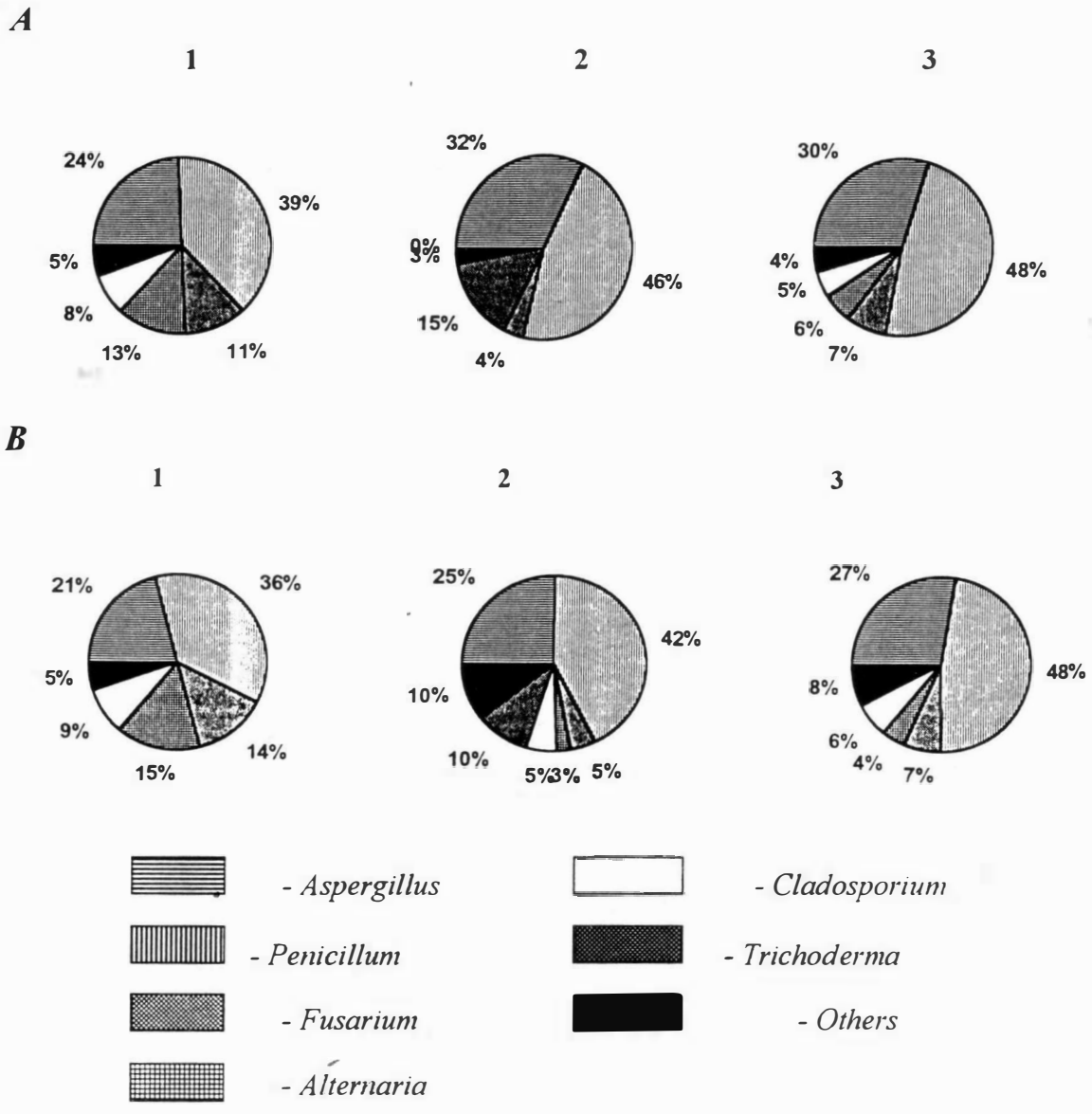

Figure 2. Percent of fungal genera colonizing pine and larch roots on seedlings treated with *Trichoderma harzianum* (isolate U) and *Pseudomonas fluorescens* (isolate 94) compared to untreated seedlings.

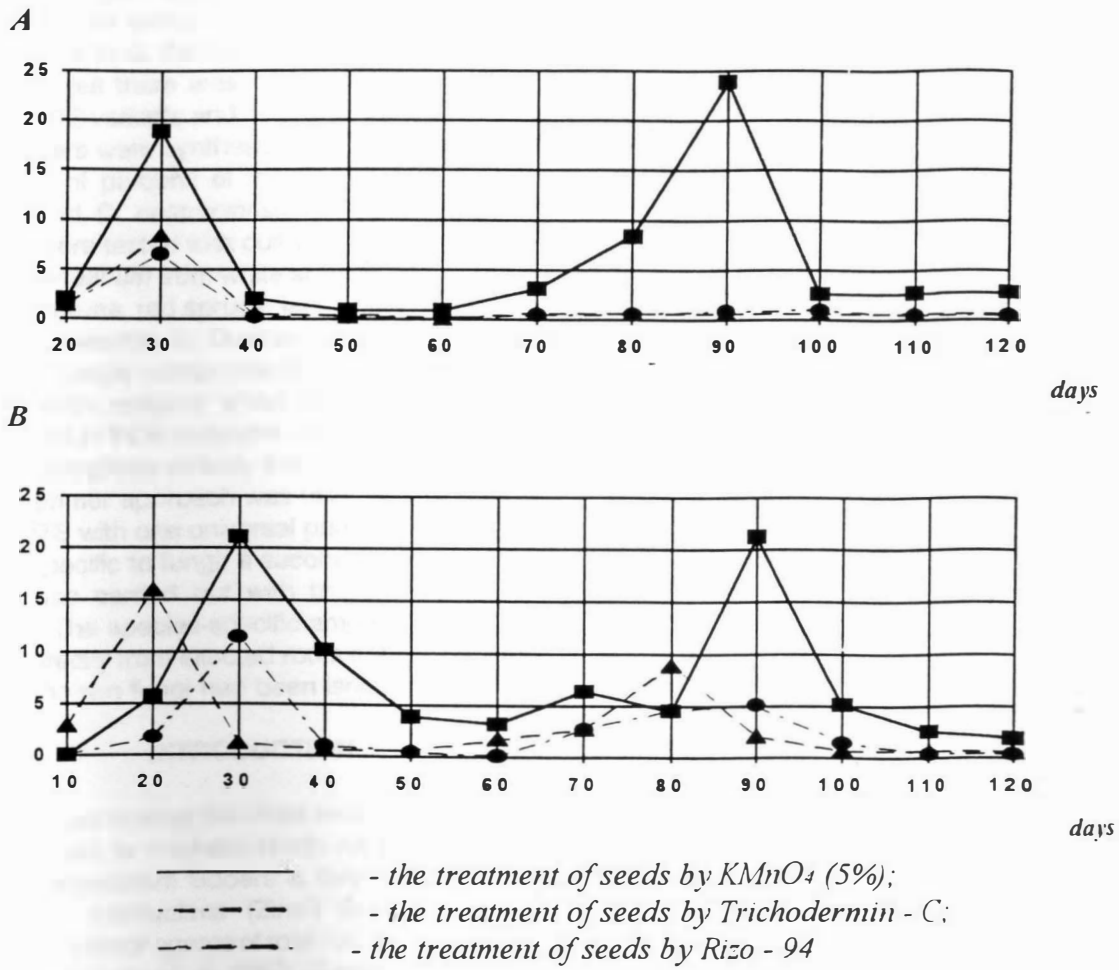

Figure 3. Effects of biological control preparations (*Trichoderma harzianum* - isolate U and *Pseudomonas fluorescens* - isolate 94) on Scots pine and Siberian larch seedling mortality in bareroot Siberian nurseries. Seedling losses calculated with the formula: $\frac{N1-N0}{N1}$ = Percent

IDENTIFICATION OF ROOT-ROT FUNGI IN NURSERY SEEDLINGS[1]

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ABSTRACT

The internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) subunit repeat was sequenced in nine isolates of *Cylindrocladium floridanum* and 11 isolates of *Cylindrocarpon destructans*. Sequences were aligned and compared with ITS sequences of other fungi in Genbank. Some intra-specific variability was present within our collections of *C. destructans* but not in *C. floridanum*. Three variants were identified, but there was no apparent association between ITS variants and host or geographic origin. Two primers were synthesized for the specific amplification of portions of the ITS for both *C. floridanum* and *C. destructans*. The species-specific primers were tested with cultures of *C. destructans* and *C. floridanum* from white spruce, black spruce, Norway spruce, red spruce, jack pine and red pine from 11 nurseries in Quebec as well as with 20 common fungal contaminants of conifer roots. No amplification resulted when the specific primers were used in PCR reactions with non-target fungi. For amplifications directly from infected tissues, a nested primer approach was used by first amplifying the ITS with one universal primer and a second primer specific to fungi; a second round of amplification was carried out with the species-specific primers. The species-specific amplicons were amplified directly from infected roots from which either one or the two fungi had been isolated.

INTRODUCTION

Root-rots are among the most serious conifer nursery diseases in northern North America. *Cylindrocladium floridanum* Sobers & Seymour and *Cylindrocarpon destructans* (Zins.) Scholten are two important causal agents of root-rots (Anderson et al. 1962; Saunders et al. 1992; Sutherland 1991). In Quebec, 262 million seedlings were inspected in 1993 for health certification; 4 million seedlings were destroyed because they did not meet health standards. Current diagnostic and detection methods involve fungal isolation and culture followed by identification using morphological characteristics. These steps are time-consuming and can lack sensitivity; for example, when aggressive contaminants prevent growth of pathogenic organisms. The objective of this study was to develop a PCR-based assay for the efficient and sensitive detection of *C. floridanum* and *C. destructans* in nursery seedlings.

MATERIALS AND METHODS

Fungal cultures were made from seedlings collected from several nurseries in Quebec. Between 4 and 16 wood chips approximately 5 x 10mm were aseptically excised from roots and root collars of seedlings showing symptoms. Chips were placed on potato dextrose agar and incubated for 10 days at 20°C. All seedlings from which either *C. floridanum* or *C. destructans* were isolated were stored in a freezer at -20°C until DNA was extracted from infected root systems. Isolates were obtained from infected nursery seedlings belonging to six conifer (*Pinus banksiana* Lamb., *P. resinosa* Ait., *Picea mariana* (Mill.) B.S.P., *P. glauca* (Moench) Voss, *P. abies* (L.) Karst., *P. rubens* Sarg.) and one broad-leaved species (*Juglans nigra* L.) from eight nurseries and three plantations situated in eight administrative regions in Quebec.

Isolates were classified to species according to their morphological, cultural, and sporulation characteristics (Booth 1966; Crous et al. 1992; Crous and Wingfield 1994; Domsh et al. 1980). Eleven isolates of *C. destructans* and 12 of *C. floridanum* that clearly satisfied the species criteria were included for sequencing and as positive controls for the species-specific primers. Isolates of 26 contaminanting fungi commonly colonizing roots were cultured and processed for DNA extraction. These included *Botrytis cinerea* Pers. ex. Fr., *Sirococcus conigenus* (D.C.) P. Cannon & Minter, *Pestalotiopsis funerea* (Desmas.) Steyaert, *Gliocladium roseum* Bounier, *Mycelium radicis atrovirens*, isolates belonging to *Fusari-*

[1] Paper presented at the third meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). Orlando-Gainesville, FL., USA. May 19-24, 1996.

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um spp. (9), *Phomopsis* spp. (1), *Coniothyrium* sp. (1), in addition to unidentified hyphomycetes (3), coelomycetes (3), deuteromycete (1), and three non-sporulating basidiomycetes. In addition, isolates identified as *Cylindrocarpon* spp. other than *destructans* were tested.

DNA was extracted according to the following protocol adapted from Zolan and Pukkila (1986). Approximately 10mg of lyophilized mycelium was mixed with diatomaceous earth (10mg) and ground in 700ul of extraction buffer (700mM NaCl, 50mM Tri-HCl [pH 8], 10 mM EDTA, 1% b-2-mercapto- ethanol, 1% cetyl-trimethyl-ammonium bromide) for DNA extraction. The mixture was incubated at 65°C for 1 hr. and extracted by adding an equal volume of chloroform-isoamyl alcohol (24:1), vortexing, and centrifuging for 5 mins. at 10,000 x g. The aqueous phase was precipitated with cold isopropanol and centrifuged for 5 mins. at 10,000 x g. The pellets were washed with 70% ethanol, air dried, resuspended in 50ul of TE8 (10mM Tris-HCl [pH 8], 1mM EDTA), and stored at -20°C until needed.

For DNA extractions directly from root collars, outer bark of the collar was removed with a scalpel and two to four strips of wood (approximately 3 x 10mm) were excised and ground in 400ul of Qiagen extraction buffer (100mM Tris-HCl, pH 9.5, 2% CTAB, 1.4 M NaCl, 1% PEG 8000, 20mM EDTA, 1% mercapto-ethanol) mixed with approximately 10mg of diatomaceous earth. Extracts were vortexed, incubated at 65°C for approximately 1 hr. extracted once with 400ul of phenol: chloroform: isoamyl alcohol (25:24:1), and centrifuged at 10,000 x g for 5 mins.. The supernatant was transferred to a new microtube, precipitated with one volume of isopropanol, and centrifuged at 10,000 x g for 5 mins.. Pellets were washed with 70% ethanol, air-dried overnight and resuspended in 20ul TE buffer, pH 8. For DNA extractions from roots, entire rootlets were cut into small pieces and processed as above.

The entire region containing portions of the small subunit (18s), both internal transcribed spacers (ITS) and the 5.8s of the rDNA repeat unit was amplified using universal primers according to the manufacturer's protocol (Gardes and Bruns 1993; White et al. 1990). The reactions were carried out on a MJ-Research PTC-60 thermal cycler (Watertown, Mass.) and consisted of an initial denaturation at 95°C for 3 mins. followed by 30 cycles of 92°C for 30 sec. 55°C for 30 sec. and 72°C for 1 min. The reaction was completed by a 10 min. extension at 72°C. PCR products were purified on a minispin column (Wizard Kit, Promega, Wis.) and sequenced with a DNA Direct Sequencing kit (Gibco-BRL, Md.) according to the manufacturer's recommendations with gP33ATP (Amersham, Ill.) labelling of the primers.

In order to conduct multiplex PCR, primers were designed to amplify PCR products of different lengths for the two target species. Specificity of the primers Dest1 and Dest4 for *C. destructans* and Flor2 and Flor5 for *C. floridanum* was tested on all *C. floridanum* and *C. destructans* DNA templates, as well as with DNA templates of the 26 common contaminants.

A nested primer PCR combined with a multiplex PCR approach was used for detection from infected seedlings. Fungi present in roots and root collars of 34 infected and 2 healthy seedlings were identified by isolation as described above. The DNA was extracted from the root collars and diluted 1:100; 1ul was pipetted in a PCR reaction using ITS1F and ITS4 primers to amplify the entire ITS region using the amplification profile described above. A second amplification with all four species-specific internal primers was conducted using as a template 1ul of PCR product from the previous fungus-specific amplification. Only 15 cycles were run with denaturation at 94°C for 20 sec., and an annealing temperature of 60°C to increase specificity. Positive (genomic DNA from the target fungus) and negative (no template DNA) controls were included in all tests.

PCR products were separated by gel electrophoresis on 1.5% agarose in TAE buffer or on 1% agarose and 1% Synergel (Certified Biotech.) in TPE buffer and visualized by UV fluorescence.

RESULTS AND DISCUSSION

All isolates of *C. floridanum* had the same ITS sequence. However, some intra-specific variability was found in the ITS sequences within our collections of *C. destructans*. Three groups were identified within *C. destructans* based on their ITS sequences. One group, Dest C, was clearly more divergent from Dest A and Dest B. There were 4 insertion/deletions (indel), 16 transitions, and 12 transversions between Dest A and B, and Dest C. There were no indels and only one transition and one transversion between Dest A and Dest B. There was also some variability within the groups. For example, there was a transversion within Dest A at position 433 and two insertions within Dest C at positions 435 (T) and 537 (A). All *C. destructans* isolates were formally identified as belonging to this species according to the following morphological and cultural characteristics: (1) chlamydospores were produced in the

mycelium and sometimes in the macroconidia, (2) microconidia (6-11 x 2-4um) zero to one-septate were present, (3) macroconidia were 1-3[4] septate, straight, cylindrical with a round apical cell, and (4) the cultures were pigmented brown to dark brown (Booth 1966).

Species-specific primers were selected in regions that were variable between, but conserved within, species. Primers Flor2 and Flor5 were designed to amplify a 328 bp PCR product starting at position 109 in *C. floridanum*. In *C. destructans*, it was difficult to target regions that were conserved within and variable between species, and that also produced a PCR fragment of a different size from *C. floridanum* so that it could be amplified in a multiplex reaction and resolved by electrophoresis. Two primers were designed to amplify a 400 bp amplicon in variants Dest A and Dest B and a 399 bp amplicon in Dest C of *C. destructans*. The mismatch at the priming site in Dest C was situated in the center of the primer Dest4; at the annealing temperature used, amplification of a product in Dest C was not prevented by this mismatch. The primers were therefore successfully designed to overcome the genetic variability present within *C. destructans*.

Specificity of the primers was confirmed on genomic DNA from all cultures of *C. floridanum* and *C. destructans*. No amplification resulted from PCR reactions using either set of primers with contaminant genomic DNA as template, including closely related species of *Cylindrocarpon*.

The first round of amplification from infected tissues using primers ITS1F and ITS4 resulted in PCR products of variable sizes, as would be expected if several fungal species were associated with decaying roots. However, the second round of amplification with all four specific primers in a multiplex PCR reaction yielded either one or two amplicons of the expected size.

Cylindrocladium floridanum was detected in 100% infected seedlings from which it was isolated. In one case, the fungus was detected in the seedlings by PCR but was not isolated. In the two healthy seedlings, the fungus was not isolated and the diagnostic PCR was negative.

Cylindrocarpon destructans was detected in 20 of 27 infected seedlings from which the fungus was isolated and two seedlings from which only *C. floridanum* was isolated. On five infected seedlings, the diagnostic PCR on DNA extracts from root collars was negative for *C. destructans*, although the fungus was isolated only from rootlets. The multiplex PCR was effective as the two expected products were amplified simultaneously in 20 reactions.

This work illustrated the potential for detecting root rot fungi directly from infected seedlings with a minimum of manipulation. This approach is particularly well suited to soil organisms that are difficult to identify or isolate due to presence of other aggressive species. In addition, some of the DNA samples were extracted from seedlings stored at -20°C for one year. This has important implications for detection on an operational basis since large numbers of samples could be frozen during the fall inspection and processed during the winter before spring outplanting.

Two obvious practical problems might limit use of this technique operationally. First is the danger of carry-over contamination. In spite of careful laboratory manipulations by trained personnel, there were occurrences of carry-over contaminations in some of our experiments and these had to be repeated with new reagents. Different means of circumventing these problems, including enzymatic digestions and UV irradiation, have been proposed and could be implemented. The second practical problem concerns the cost of such analyses. Although it would be impossible to carry out routine diagnostics using PCR on nursery seedlings, it could be possible to use this technique as an aid in identification in difficult cases. Another prospect would be use of bulk samples followed by a quantification assay to determine the proportion of infected seedlings. We are currently developing colorimetric assays to achieve this goal.

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LITERATURE CITED

Anderson, N., D.W. French, and D.P. Taylor. 1962 Cylindrocladium root rot of conifers in Minnesota. Forest Science 8:378-384.

Booth, C. 1966. The genus *Cylindrocarpon*. Mycological papers 104. Commonwealth Mycological Institute. 56p.

Crous, P.W., A.J.L. Phillips, and M.J. Wingfield. 1992. Effects of cultural conditions on vesicle and conidium morphology in species of *Cylindrocladium* and *Cylindrocladiella*. Mycologia 84:497-504.

Crous, P.W., and M.J. Wingfield. 1994. A monograph of *Cylindrocladium*, including anamorphs of *Calonectria*. Mycotaxon 51:341-435.

Domsh, K.H., W. Gams, and T. Anderson. 1980. Compendium of soil fungi. Academic Press, London. 859p.

Gardes, M., and T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113-118.

Saunders, J.E., J. Juzwik, and R. Hutchison. 1992. Outplanting survival of Cylindrocladium root rot affected black spruce seedlings. Canadian Journal of Forest Research 22:1204-1207.

Sutherland, J.R., S. Greifenhagen, J. Juzwik and C. Davis. 1991. Diseases and insects in forest nurseries in Canada. *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada. Pacific and Yukon Region. Information Report BC-X-331. pp. 25-32.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M. (ed.), PCR protocols: A guide to methods and applications. Academic Press, San Diego. pp. 315-322.

Zolan, M.E., and P.J. Pukkila. 1986. Inheritance of DNA methylation in *Coprinus cinereus*. Molecular and Cellular Biology. 6:195-200.

EFFECT OF METHYL CIS-7-OXO-DEISOPROPYLDEHYDROABIEATE ON SPORE GERMINATION OF BOTRYTIS CINEREA: OBSERVATIONS BY TRANSMISSION ELECTRON MICROSCOPY[1]

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ABSTRACT

Methyl *cis*-7-oxo-deisopropyldehydro- abietate (MC7OD) is a resin acid derivative from pine rosin, an important natural resource in Portugal. Conidia of *Botrytis cinerea*, isolated from *Pinus pinaster* seedlings, were treated with MC7OD and observed for morphological alterations by light and transmission electron microscopy (TEM) after 24 hrs. incubation. Effects of MC7OD at concentrations of between 0.003% - 0.5% were evaluated on spore germination. In non-treated cultures, about 100% of the conidia germinated after 24 hrs. However, only some of the spores treated with MC7OD germinated and some of these had shorter germ tubes than untreated spores. Some treated spores did not germinate even though they appeared normal morphologically; other spores lysed following cell wall rupture. TEM indicated that morphological alterations caused by MC7OD occurred mainly to organelle membranes. Autophagic vacuoles also became visible and strongly-stained lipids accumulated inside vacuoles at the cell periphery. These observations may be related to *B. cinerea* susceptibility to MC7OD toxicity. Morphological modifications in membrane systems caused by MC7OD may interfere with metabolic functions necessary for spore germination.

INTRODUCTION

Antimicrobial activities of dehydroabietic acid and similar derivatives have been previously described (Hendricks et al. 1979). Methyl *cis*-7-oxo- deisopropyldehydroabietate (MC7OD) is an important resin acid derived from pine rosin, which is extensively collected in Portugal. Fungicidal activity of MC7OD was detected *in vitro* and *in vivo* against *Botrytis cinerea* Pers. ex Fr. (Feio et al. unpublished) and *Lophodermium seditiosum* Minter, Staley & Millar (Feio et al. 1995, patrhogens of several *Pinus* spcies in nurseries.

MATERIALS AND METHODS

Botrytis cinerea was isolated from *Pinus pinaster* Ait. seedlings and grown on malt extract agar (MEA). MC7OD was prepared at technical grade according to the procedure described by Gigante et al. (1991). This compound was dissolved in acetone (P. A. Merck) in order to obtain concentrations of between 0.003-0.5% (w/w, product/culture-medium). 0.1 ml of a spore suspension of *B. cinerea* at 10^5 spores/ml was inoculated onto MEA amended with MC7OD in petri dishes. Unamended media containing acetone served as a control. Spore germination was estimated after 24 hrs. incubation. Relative inhibition (IR) was calculated using the formula:

$$IR = \frac{(No - N) \times 100}{No,}$$

where:

No = number of germinated spores in control cultures.
N = number of germinated spores in cultures treated with MC7OD

Spores treated with MC7OD at a concentration of 0.06% were chosen for TEM observation. Conventional fixing and staining methods were used (Reynolds 1963): fixation by Karnowsky buffer and staining by osmium tetroxide, inclusion on Spurr resine and re-staining with Au/Pb.

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RESULTS AND DISCUSSION

Light Microscopy:

Effects of MC7OD at concentrations between 0.003-0.05% on *B. cinerea* conidial germination are summarized in Figure 1. Spore germination within control cultures approximated 100% after 24 hrs. However, in the presence of MC7OD at concentrations between 0.125-0.5%, spore germination was greatly inhibited. Some spores only partially germinated with reduced length of germ tubes. Other spores did not germinate even though they appeared normal morphologically. A few spores lysed following rupture of the cell walls.

Transmission Electron Microscopy:

Spores germinating on MEA unamended with MC7OD (controls) had nuclei, mitochondria, and vesicles with well-delimited membranes (Figure 2). Accumulation of intracellular lipids was not observed in these spores. However, spores exposed to MC7OD contained autophagic vacuoles and most other membraneous organelles were not detectable. Strongly-stained lipid accumulations were evident inside vacuoles near the spore periphery. Increasing lipid accumulations in microorganisms may be a response to toxic compounds (Bishop and Jeves 1984). Therefore, increasing lipid accumulations in *B. cinerea* spores responding to MC7OD may be related to the toxic character of that resin acid. It is possible that MC7OD is retained by intracellular lipids, since that toxic compound has liposoluble characteristics. Also, osmium tetroxide used in post-fixation preparations of samples may oxidize MC7OD and strongly stain lipids.

Conclusions:

We believe that MC7OD disrupts *B. cinerea* condial germination by disrupting cellular organelles and interferring with normal metabolic processes involved in germination. This results in reduced and abnormal germination as well as destruction of some spores. This information may be useful in improving control measures for this important nursery pathogen. Because MC7OD seems effective in disrupting spore germination at such small concentrations, perhaps it can be used alone or in combination with other chemicals to treat nursery crops prone to damage by *B. cinerea*.

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LITERATURE CITED

Bishop, D.C. and M.T. Jeves. 1984. Ultrastructure of induced iprodione insensitive isolates of *Botrytis cinerea*. Transactions of the British Mycological Society 83:507-510.

Feio, S., N. Fonseca, B. Gigante, and M.J. Marcelo-Curto. 1995. Combate ao parasita *Lophodermium seditiosum* em viveiro pelo uso do composto cis-7 oxo-desisopropil-desidroabietato de metilo. Silva Lusitana 3(1):23-36.

Gigante, B., A.M. Lobo, S. Prabhkar and M.J. Marcelo-Curto. 1991. New selective synthesis of oxidized diterpenic resin acid derivative. Synthetic Communications 21 (18-19): 1959-1966.

Hendricks, L.M., R. Ekman and K. von Weissenberg. 1979. Bioassay of some resin and fatty acids with *Fomes annosus*. Acta Acad. Abo. Ser. B. 39:1-7.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. Journal of Cell Biology 17: 208-212.

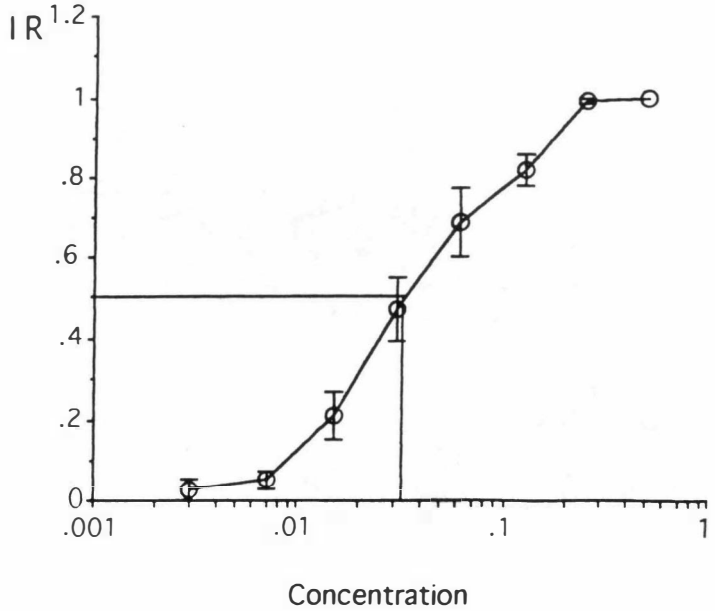

Figure 1. Effect of methyl *cis*-7-oxo deisopropyldehydroabietate on *Botrytis cinerea* condial germination. IR = relative inhibition.

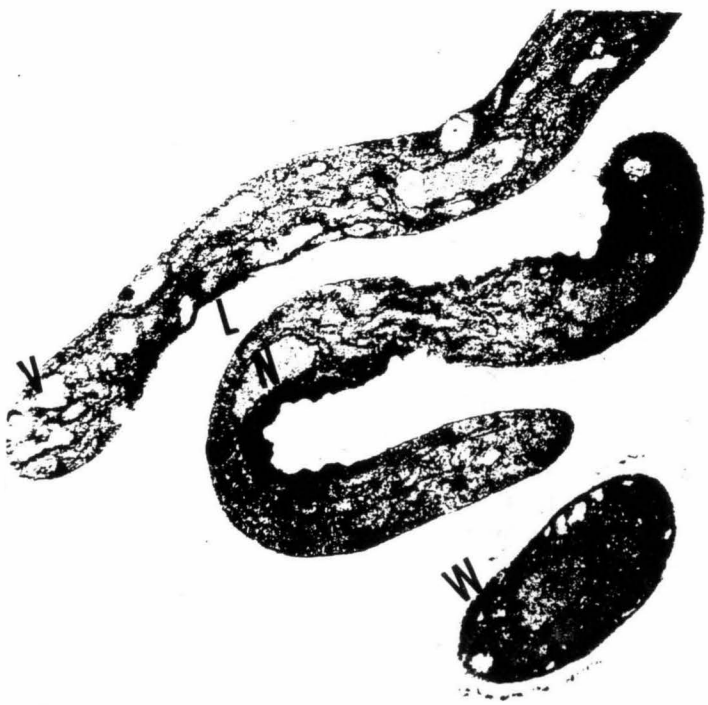

Figure 2. Transmission electron micrograph of longitudinal sections through young hyphae of a control culture of *Botrytis cinerea*. N = nucleus; W = cell wall; V = vacuole. (x6800).

Figure 3. Transmission electron micrograph of longitudinal and transverse sections of young *Botrytis cinerea* hyphal cell treated with methyl cis-7-oxo- deisopropyldehydroabietate. Note lipid accumulation (L) at the cell periphery. (x5000).

DISEASE AND INSECT RESPONSES TO INTENSIVE CULTURE OF SOUTHERN PINES[1]

G.M. Blakeslee[2]

INTRODUCTION AND STUDY METHODS

Nutritional deficiences, inter-specific competition, and moisture deficiency are hypothesized as critical limiting factors in preventing slash (*Pinus elliottii* Engelm.) and loblolly (*P. taeda* L.) pines from reaching their biological growth potential. This study[3], designed as a 2x2x2 factorial with three replications representing differing moisture regimes, evaluates this hypothesis. The study site lies within the natural range of both pine species, but is on a site typically occupied by and managed for slash pine. Fertility was either unaltered or enhanced with a complete balanced fertilizer with repeated applications; inter-specific competition was either unaltered beyond that occurring with standard site preparation or was completely and continuously controlled. Moisture was treated through recognition of the naturally occurring range of moisture regimes on the site, the naturally wetter portion of which received supplemental irrigation at a level which attempted to eliminate cumulative deficits when compared to the norms for the area. The slash pine families utilized in the study had been improved for growth traits and resistance to fusiform rust. Loblolly pines represented progenies of untested selections, most of which derived from the southernmost portion of the species range. North central Florida is generally a low risk area for fusiform rust.

Environmental conditions are known to affect the occurrence of many biotic agents capable of affecting tree health. Because the treatments in this study spanned an exceedingly wide range of culturally modified environmental conditions, it offered considerable opportunity to observe resultant disease and insect responses. Accordingly, during the first 5 years of growth, we made annual observations to evaluate the occurrence of disease and insect attacks on the 900+ trees within the study.

PRELIMINARY RESULTS AND OBSERVATIONS

At age 5, using plot volume as a measure of stem growth, slash pine substantially out-grew loblolly pine in the untreated areas which represented cultural treatments similar to those used in conventional practices for slash pine on sites such as this. Slash pine showed marginal advantage over loblolly pine when nutrients were supplemented or when inter-specific competition was controlled; however for both species, growth greatly exceeded that accumulated in the unaltered plots. When nutrition was supplemented and competition was simultaneously controlled, growth was greatly increased, showing a nearly additive effect of the individual treatments. However, in this maximum treatment, slash pine growth ceased to exceed growth of loblolly pine.

Four biotic agents were prominent amongst those agents affecting tree health: the pitch canker fungus [*Fusarium subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas f. sp. *pini*] , the pitch moth/coneworm [*Dioryctria amatella* (Hulst.)], the fusiform rust fungus [*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Hedge. & N. Hunt) Burdsall & G. Snow], and tip moths (*Rhyacionia* spp.). Of these, pitch canker, pitch moth and fusiform rust were the most destructive. Although tip moth attacks were extremely common on loblolly pine, they were less damaging.

In both pine species, pitch moth incidence on a per tree basis increased as cultural intensity increased. Pitch moth attacks were uncommon (<5%) on control plots, somewhat higher (ca 5-10%) on single treatment plots, but markedly increased (ca 20-30%) in the presence of both nutritional supplement and competition control. Overall, loblolly pine evidenced fewer pitch moth attacks

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[3] This research was conducted by scientists of the USDA Forest Service under the auspices of IMPAC (Intensive Management Practices Assesment Center), and by scientists from the University of Florida. The extensive support of JSC/Container Corporation of America is gratefully acknowledged.

did slash pine. Many pitch moth attacks occurred in combination with fusiform rust infections, although many points of infestation occurred in the absence of any predisposing infection or injury.

The incidence of fusiform rust stem infections was relatively low overall, although loblolly pine was more commonly infected, especially in the combined treatments where ca 20% of the trees were infected. In loblolly pine, absence of competing vegetation strongly favors proliferation and retention of lower branches. This characteristic may favor infection and aid the obligate parasite in reaching the main stem via branch infections.

On slash pine, pitch canker was the most common and damaging biotic agent. Although infrequent on check plots, the incidence of pitch canker attacks on slash pine stems increased as cultural intensity increased, eventually involving one of every three trees on the plots receiving the combined treatments. Many of these infections occurred independently from any other attack, however multi-agent attacks were common. When simultaneously occurring with fusiform rust and/or pitch moth attacks, pitch canker infections greatly accentuated the damage caused by either agent alone. Incidence of main stem pitch canker infections on loblolly pine was markedly lower than that on slash pine, although pitch canker infection of growing shoot apices attacked by tip moths was more common.

The lower overall rust infection of slash pine was expected as the selected families were improved for resistance to fusiform rust. Had non-improved stock been used or had the study been located in a high fusiform rust risk area, higher infection levels of both species would have been expected. The southern origin of the loblolly pine families may partially explain lower pitch canker incidence in that species as a similar source-resistance relationship has been reported in slash pine. Pitch canker resistance was not considered in selection of slash pine families, although family-related differences in infection became evident as the study progressed. The synergistic effects of the multi-agent complexes frequently induced tree mortality, gross stem deformation, and may be responsible for the productivity decline observed in slash pine.

Tree growth, stand development, and pest impacts since age 5 are currently being assessed. To date, increased mortality and deformity of slash pine due primarily to the cumulative impacts of pitch canker, pitch moth, and fusiform rust are apparent in the combined treatment plots. Growth of pest free trees has been significant, rivaling growth rates attained in the southern hemisphere where these species are commercially grown. Also apparent is the increase in pitch canker occurrence in the control and single treatment plots. It is hypothesized that these increases in pitch canker incidence reflect the influence of increasing competitive stress that occurs with stand development. Although incomplete, current data suggest a leveling of disease incidence across treatments in slash pine and stablility of expressed resistance in combined treatment plots.

These observations demonstrate the potential importance of biotic agents in affecting tree health and in doing so, suggest the relevance of their role in limiting the productive capacity of these species under conditions designed to alleviate potential restrictions attributable to abiotic factors. The dynamics of multi-pest-host-environmental interactions are imperfectly understood. However, studies such as these should provide insight into these dynamics as expressed under field conditions over extended time periods.

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